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DEPARTMENT OF AGRICULTURE

MYSORE STATE

SPIKE DISEASE OF SANDAL

BY

LESLIE C. COLEMAN, M.A., PH.D.

Director of Agriculture in Mysore

WITH NINETEEN PLATES

BANGALORE:
PRINTED AT THE GOVERNMENT PRESS
1917

PRICE ONE RUPEE.

SPIKE DISEASE OF SANDAL

FIG. 1.
A Healthy Sandal Tree.

FIG. 2.
Sandal Tree fully attacked by Spike Disease.

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FOREWORD.

THE investigation of the disease known as Sandal Spike has been on the programme of the Mysore Agricultural Department for many years. Lack of staff and pressure of work associated with the organization and rapid expansion of departmental activities, however, prevented its being taken up in a methodical manner till about three years ago. Even during this latter period I have been able to devote only a comparatively insignificant portion of my time to the subject.

The recent phenomenal increase in the value of Sandal has led to a very marked growth in interest in this disease, and although our investigations have proceeded but a short way on the road to elucidating its cause and the means for its control, I have thought this an opportune time to publish the results that have been already obtained.

While this report, dealing as it does with a most obscure disease, is not intended as a popular bulletin, I have tried to make it as clear as possible and to keep it free from a mass of technical detail which would, in any case, hardly serve any useful purpose. As it will probably be of special interest to Forest Officers in India, many of whom may not have access to literature on this and related subjects, I have given as full an account as I could, with the materials at my disposal, of the work that has been done on it in the past, and have, in addition, discussed briefly diseases of a similar character which have been investigated in other parts of the world.

The report is intended simply to clear the ground for more continuous and co-ordinated work in the future. In this connection I wish to lay stress on the importance of

associating all agencies which are able to investigate any branch of the subject, whether in the field or in the laboratory, in the closest manner possible. The importance of the *rôle* which Forest Officers can play in the investigation of this disease must be apparent, and I wish to express my appreciation of the cordial manner in which these officers, both inside and outside the State, have assisted me with advice and information.

The bulk of the microscopic and other experimental work on the botanical side has been carried out under my direction by Mr. M. J. Narasimhan, B.A., Assistant Mycologist. On the chemical side I have to acknowledge the assistance of Mr. H. V. Krishnayya, B.A., Chemist, Agricultural and Geological Departments, Mr. Sankar Rao Badami, M.A., Assistant Chemist, and Mr. N. Sampatiengar, Senior Assistant Chemist. Mr. Sankar Rao Badami has more especially devoted his time and attention to the subject.

The subject is both important and difficult. The Government of His Highness the Maharaja of Mysore are fully alive to this, and have recently sanctioned the appointment of a specially trained officer to be associated with me in its further investigation.

LESLIE C. COLEMAN.

BANGALORE,
May, 1917.

SPIKE DISEASE OF SANDAL.

THE first report of the disease of the sandal tree, known as "Spike," appears in a most interesting and valuable memorandum "On the Future Supply and Culture of Sandal in Coorg," by Mr. C. McCarthy, then Deputy Conservator of Forests in Coorg. This memorandum was submitted to the Secretary to the Chief Commissioner of Coorg in December 1899, but has never been published, which is the more to be regretted, as it contains one of the ablest and clearest descriptions of the disease as it occurs in the field that has been written.

The first published record of the disease is contained in the report of the Forest Administration in Coorg during the year 1898-99, and from that time references to it are frequent.[1] From these early reports it is apparent that the disease must have been existent for several years prior to 1898. The first area known to be infected was that in the neighbourhood of Fraserpet on the Coorg-Mysore boundary. Whether the disease originated in Coorg and spread from there to Mysore or whether it appeared simultaneously on both sides of the boundary is uncertain. In any case we know that the spread of the disease was fairly rapid, for in March 1903[2] Mr. Muthannah, then Officiating Conservator of Forests in Mysore, reported it as far east as Yelwal on the Mysore-Fraserpet road. In his Annual Report for 1903 this officer expresses the opinion that it first made its appearance in Mysore about ten years previously, but he does not state on what data this opinion is based.

[1] References to the appearance and spread of Spike Disease in Mysore and Coorg are to be found in the following Government publications:—

i. McCarthy, Progress Report of Forest Administration in Coorg for 1898-1899. References are to be found in all subsequent Coorg Forest Reports.

ii. Muthannah, Progress Report of Forest Administration in the Mysore State for the twelve months ending 30th June 1903. References are also to be found in practically all subsequent Mysore Forest Reports.

iii. Government of Mysore Forest Proceedings. No. 12-21-112-21 dated 30th July, 1903. This contains, among other papers, Notes by Dr. Butler and Mr. Barber, detailing results of their scientific investigations and Notes and Reports by Mr. M. Muthannah, Officiating Conservator of Forests in Mysore and other forest officers, giving the results of field observations.

iv. M. Muthannah, Selections from Reports and Notes on Spike Disease of Sandal, Mysore Government Publication, 1906. Will be referred to hereafter as Mysore Forest Selections, 1906.

[2] *See* Government of Mysore Forest Proceedings noted above, p. 13.

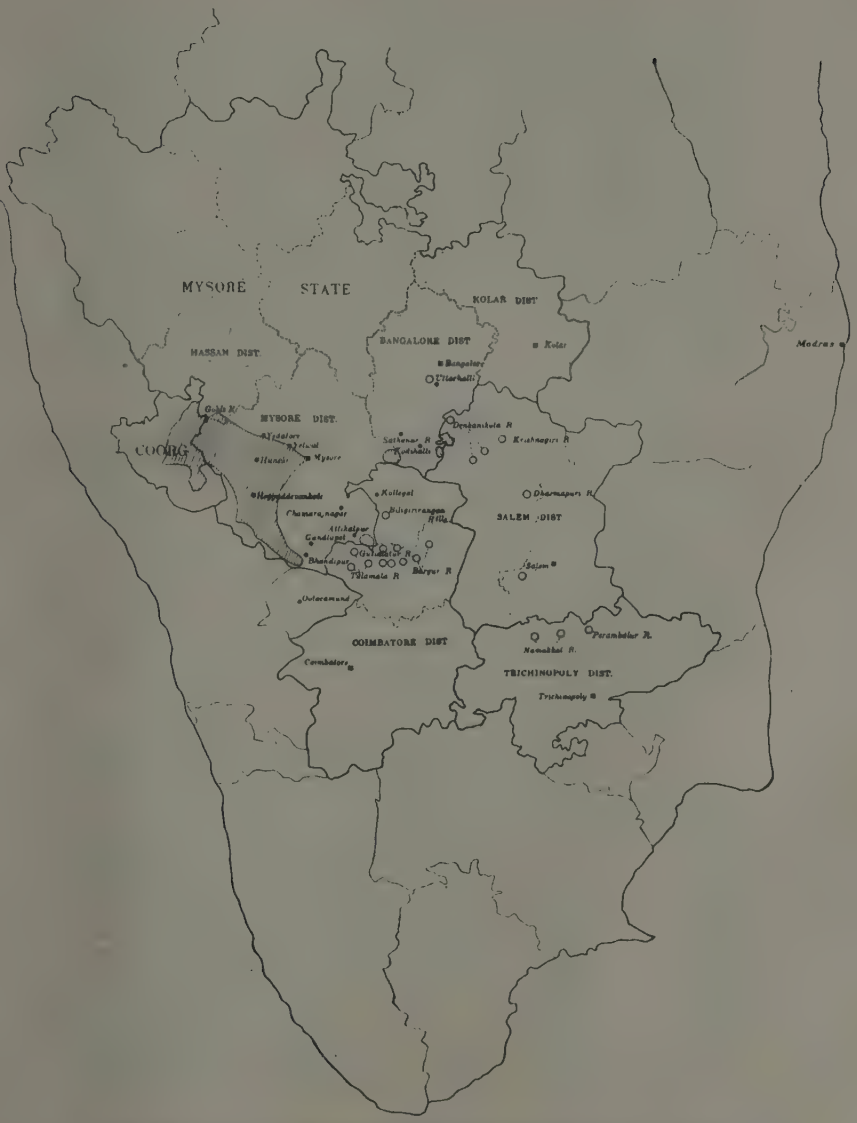
In his Annual Report for 1903-04, Muthannah writes as follows with regard to the spread of the disease :—

"The disease has spread over a very large area in the Sandal belt of this district (Mysore District). The whole of Hunsur Taluk has been overrun by the disease and chiefly in areas infested by lantana. The bordering villages of the Mysore and Heggaddevankote Taluks are also infected by the disease. It has lately made its appearance in the Mysore Town and four affected trees have already been discovered and removed. A belt about three miles wide and extending over a length of about forty miles is sporadically affected in the Heggaddevankote Taluk. In the Gundlupet and Chamrajnagar Taluks also the disease has made its appearance and in the latter taluk it has spread gregariously over about two square miles and a very large number of young saplings have been affected. In other places, the disease has appeared only in a sporadic form."[1]

In the following year, he reports the whole of Heggaddevankote Taluk infected, while, in his report for 1905-06, he states that the disease had spread in Chamrajnagar and Gundlupet Taluks at an alarming pace. In the report for 1908-09, he notes the appearance and spread of the disease in the Basavanbetta Forest of the Mysore District on the borders of Bangalore District, while the next year it had appeared in Bangalore District itself. In the report for 1912-13, the disease is said to have spread in Bangalore and Mysore Districts, while the statement is made that during the previous fourteen years the disease had wiped out ninety per cent of the sandal trees in the western half of the Hunsur Taluk, one of the best sandal tracts in the State. In 1914, it had appeared in the village of Uttarhalli, only six miles from Bangalore.

The above record of the spread of the disease in Mysore is, in all probability, not complete. The account given, however, shows that the disease has, during the past sixteen years, spread over practically the whole of the southern part of the State from west to east and that it has been at the same time spreading northward. There are still large sandal areas in the State uninfected, but, as far as we can see, there seems to be nothing in the way of these areas becoming infected also, in which case the future of sandal as a source of forest revenue would be seriously threatened. Outside of Mysore, also, the disease has appeared in many places. The accompanying map (text-figure 1), which has been compiled on data collected from

[1] Progress Report of Forest Administration in the Mysore State for the twelve months ending 30th June, 1904, Appendix C.



TEXT-FIGURE 1.—MAP OF SOUTH INDIA, showing area attacked by Spike Disease. The large areas are indicated by diagonal shading. The smaller areas are indicated by circles. In this case, the size of the circles has no reference to the size of the areas infected. For instance, the circle connected with Salem Town represents a single tree. In the case of the circles, the forest ranges are indicated in which the attack has appeared. The administrative areas to which the disease is at present restricted, *viz.*, Mysore State, Coorg and Salem and Trichinopoly Districts in Madras Presidency, have been marked off by somewhat heavier lines.

all the sources available, shows the present known distribution of the disease.[1] From this it will be seen that about three-fourths of the sandal area in Coorg is infected. Coming to Mysore State, as already stated, the greater part of the Mysore District must be considered as infected. It is true that there are large areas in the district where the disease has not been found, but it is probable that a careful village to village search would reveal diseased trees in many parts of the areas which are shown on the map as free. In the Hassan District, there is an area in the Arkalgud Taluk very badly attacked, while, in Bangalore District, there are several attacked areas, namely those in Kankanhalli Taluk and in the neighbourhood of Uttarahalli Village, six miles from Bangalore.

In the Madras Presidency there are a number of infected areas, but these are, for the most part, still small, and, what is more remarkable, are situated at long distances from each other. We find a similar apparent discontinuity of the infected areas in Mysore.

As is well-known, Sandal has formed for many years, and still continues to form, the chief source of Forest Revenue in Mysore. The following table, which has been compiled from figures contained in the Annual Reports of the Forest Department, shows clearly the great importance of the Sandal tree to the State.

Year.		Revenue. Rs.
1899-1900	...	9,35,820
1900-1901	...	7,64,294
1901-1902	...	9,81,747
1902-1903	...	10,03,351
1903-1904	...	10,35,687
1904-1905	...	12,19,021
1905-1906	..	12,64,791
1906-1907	...	11,18,219
1907-1908	...	14,20,842
1908-1909	...	9,24,558
1909-1910	...	9,31,875
1910-1911	...	11,75,009
1911-1912	...	12,49,034
1912-1913	...	22,88,114
1913 1914	...	20,62,618
1914-1915	...	3,14,819
Total Rs.	...	1,86,89,799

The average Revenue from sandal in Mysore for the sixteen years ending with 1914-15 was over Rs. 11,50,000 while in two years during the period it exceeded twenty lakhs. The sandal revenues in Coorg and in Madras Presidency, while much less than that in Mysore, are still important. During the period above mentioned the average annual revenue from sandal in Coorg was about Rs. one lakh, while in Madras Presidency the average for the ten years, 1905-06 to 1915-16, was slightly less.

[1] For the information upon which this map is based, I have to thank many of the Forest Officers of South India. Among these, I should like to mention Mr. M. G. Rama Rao, Conservator of Forests in Mysore, Mr. H. Tireman, Deputy Conservator of Forests, Coorg, Mr. P. M. Lushington, Conservator of Forests, Southern Circle, Madras, Mr. C. McCarthy, Conservator of Forests, Western Circle, Madras, and Mr. C. C. Wilson, Special Working Plans Officer, Madras.

An attempt to compute the actual losses that have resulted from this disease is confronted with many difficulties.[1] In the first place, the actual value of a sandal tree is difficult to estimate, more especially as the value of the wood has increased very greatly during recent years. Thus the average price for sandal during the ten years ending 1903 was Rs. 416 per ton while the average price for the ten years ending 1913 was Rs. 512 per ton. The prices since 1913 have shown an astonishing increase, averaging Rs. 1,067 per ton in 1913-14, Rs. 932 per ton in 1914-15 and about Rs. 2,000 per ton in 1915-16. Whether these high prices will persist, remains to be seen, but the fact that sandal is now worth over four times as much as it was at the time when Spike Disease first came under notice, shows that any estimate that could have been made at that time would be hopelessly incorrect in the light of present prices.

In estimating the amount of damage done by Spike it is necessary to estimate not only the outturn from trees at a given age, but also to estimate the average age at which trees are killed by Spike. This is an extremely difficult thing to do. As will be pointed out later in this report, it was, at the beginning, supposed that young trees were not attacked, but this was soon found not to be the case. Muthannah, in his report for 1903-04, already quoted above, expressly notes that during that year a very large number of saplings had been attacked in the Gundlupet and Chamrajnagar Taluks, and my own observations and that of others indicate that young trees are attacked just as readily as old ones. Mr. Tireman, in a letter to me, has given ten years as a rough estimate of the average age of attacked trees; and failing any more accurate estimate, I propose using that figure.

The accompanying table (Table No. 1) gives a summary of the information which is available on which to estimate the value of trees killed by Spike. As will be seen, I have

[1] My chief sources of information for calculating the losses caused by Spike Disease are the following:—

(1) Mysore Forest Reports, giving a Record of the trees removed between 1904-10 in Mysore on account of the disease.

(2) Letter from Mr. H. Tireman, giving statistics of trees removed in Coorg on account of the disease between 1900 and 1916 and estimates as to losses.

(3) P. M. Lushington, Notes on Sandal, Indian Forester, Vol. XXX, p. 14, 1904.

(4) Rao Sahib M. Rama Rao, Notes on Sandal, Indian Forester, Vol. XXX, p. 251, 1904.

(5) Rao Sahib M. Rama Rao, Tirupattur Sandalwood Working Plan, Salem District, Proceedings of the Board of Revenue, Madras, Forest No. 172, of the 20th August 1904.

(6) Information kindly placed at my disposal by Rao Sahib M. Rama Rao, giving unpublished results of his investigations.

TABLE 1.—SUMMARY OF DATA WITH REFERENCE TO MEASUREMENTS, WEIGHTS AND VALUES OF SANDAL TREES.

Locality	Girth limits	Average girth	Average age of trees calculated on girth growth of $\frac{22}{35}$ inches per year	Total No. of trees	Total weight scented wood	Average weight per tree	Value per ton	Average value per tree at 10 years age. Interest at 4 per cent compounded
		In ins.	Years.		lbs.	lbs.	Rs.	Rs. a. p.
Coimbatore District ...	16 inches upwards.	22	35	1,346	71,388	53	800	7 0 0
Javadis, 1902-03, 1903-04, Salem District.	16 inches to 60 inches.	40	Approximately 65.	294	1,00,904	343	800	14 12 0
Namakal Kollaimallis, Salem District, 1900.	19 inches to 52 inches.	30·5	Approximately 50.	105	11,075	105·5	800	7 12 0
Melagiris, Salem District, 1901-02.	19 inches to 42 inches.	27	Approximately 45.	115	9,530	83	800	6 12 0

taken a maund of 28 lbs. (one-eightieth ton) to be worth Rs. 10 (Rs. 800 per ton). This is considerably lower than the prices immediately before the war and is not half the price obtained for sandal during 1915-16. It would be obviously incorrect to take the prices which reigned from 1900 to 1912 as a standard, because, taking the sandal killed by Spike in the past to have been, on an average, ten years old, this sandal would not, had it lived, have been ready for exploitation till about 1930, when the prices seem likely to exceed Rs. 800 per ton.

One of the first difficulties in the way of estimating losses in sandal is the lack of information as to the age of trees of a given girth measurement. As far as I have been able to ascertain, the only knowledge on this subject is that obtained through the counting of the annual rings. There is a difference of opinion among Forest Officers as to whether an annual ring in sandal really represents definitely a year's growth. The only instances that I have been able to come across where annual rings do not represent a year's growth are in cases where, through defoliation, trees are stimulated to put out a second flush of leaves, in which case a second ring may be formed in the same year.[1] The age of a tree in years can therefore be hardly more than the number of annual rings though it may be less. The results of careful measurements made by Rao Sahib Rama Rao give, on an average, a radial increase of one inch for every ten annual rings. This represents an annual girth growth of 22-35 inches which I propose to take as a basis for calculating the age of trees from their girth.

The table gives the results of my computation. In estimating the average value of trees at ten years I have allowed for compound interest at 4 per cent and in estimating the average age of the trees exploited I have taken round numbers which are, in all cases, somewhat higher than the computed figures.

It is interesting to note that, although the first lot of figures given are computed from a table published by Mr. Lushington and the last two from those furnished by Mr. Rama Rao, they give values for the trees at ten years which correspond quite closely. The figures for the Javadis, also taken from Mr. Rama Rao's report, are, on the other hand, very much higher.

[1] *See* Jost, Vorlesungen über Pflanzenphysiologie, 2te Aufl. 1908, p. 425 and Haberlandt, Physiological Plant Anatomy, trans. from 4th German Edition, 1914, p. 681.

There is another correction to be made, and that is to allow for trees which would have died from other causes had they not been attacked by Spike. Mr. Tireman suggests that twenty per cent should be deducted to make this correction. If we do this, we get a value for the spiked trees which lies between Rs. 5 and slightly over Rs. 6, if we exclude the high value of Rs. 14-12-0 per tree from the calculation. We are then, I think, safe in putting the value of the losses from Spike Disease at Rs. 5 per tree, even if we make allowance for the revenue that may have been derived from such of the trees as were of exploitable age at the time they were attacked as well as for losses from driage, which Mr. Rama Rao puts at about ten per cent. Between the years 1903 and 1910, during which time there was an attempt to check the disease in Mysore by uprooting attacked trees, there is a record of 7,00,000 trees having been uprooted. If we take these trees at an average valuation of Rs. 5, we get a loss of Rs. thirty-five lakhs. It is certain that in these uprooting operations a number of diseased trees must have been left unremoved, but these need not be taken into account.

The average annual loss for the eight years during which uprooting operations were carried on thus exceeds four lakhs. In Coorg about three and a-half lakhs of trees have been uprooted during the past sixteen years. This again represents a loss of over Rs. one lakh per annum. What the losses in Madras Presidency have been I do not know, but it seems quite probable, considering the area over which the disease has spread, that a lakh of rupees' worth of damage is being done annually in that Presidency at present. We shall, therefore, not be exceeding the mark if we estimate the annual losses from Spike disease during the past ten years at between five and six lakhs rupees.

As has already been pointed out, if the disease continues to spread, not only are these losses likely to be maintained but the position of sandal as a source of revenue will be seriously threatened. In this connection may be pointed out the important fact that, in some of the areas which have suffered worst from the disease (notably parts of Hunsur Taluk in Mysore District), it has become difficult to get sandal re-established, as young saplings contract the disease long before they have reached an oil-yielding size.

The external symptoms of the disease have been well described by McCarthy,[1] Barber[2] and Butler[3] in their reports, so it is not necessary to deal with this side of the subject in detail. The most striking feature is undoubtedly the decrease in the size of the leaves which is accompanied by a shortening of the internodes so that the leaves become very closely crowded together on the leaf-bearing branches. (See Frontispiece, Fig. 2.) As pointed out by McCarthy in his original report, this does not appear, usually at least, all over the attacked tree at the same time, but shows itself on a few of the branches (see Plate I, Fig. 1), the symptoms gradually spreading to the whole tree. At the same time the leaves display a tendency to stand out stiffly from the branch thus giving rise to the somewhat spike-like appearance of the affected branches, which led McCarthy to apply the term "Spike" to the disease. As the disease advances, the fresh leaves produced become smaller and smaller. Thus normal fully developed leaves of Sandal are about three inches long and one inch wide, whereas in the last stages of spike, they have been found as small as one-third inch long by one-sixteenth inch wide. At times we do not find this gradual reduction in the size of the leaves; the first twigs showing spike bear, in such cases, leaves which are quite small. Plate I. Fig. 2 shows a tree in an advanced stage of the disease where all the leaves have been reduced to very small dimensions.

Another striking feature in connection with the disease, which was noted by McCarthy, is the continuous growth in a tree or parts of a tree attacked by Spike. While sandal trees can be found putting out new leaves in practically every month in the year, this usually occurs where branches have been lopped or broken off, and there is normally a certain fairly well-marked periodicity in leaf development. The great majority of young leaves begin to grow in February or March and reach their full development in the following August and September.[4] In the case of spiked branches, however, there is none of this periodicity, fresh flushes of new leaves being produced

[1] McCarthy, Unpublished Memorandum on the Future Supply and Culture of Sandal in Coorg, 1899.

[2] Barber, Report on Spike Disease of Sandalwood Trees in Coorg. Indian Forester, Vol. XXIX, p. 21, 1903. Also published in Mysore Forest Selections, p. 11, 1906.

[3] Butler, Report on Spike Disease among Sandalwood Trees, Indian Forester, Vol. XXIX, (Appendix Series), 1903. Also in Mysore Forest Selections, p. 1, 1906.

[4] This statement applies particularly to the eastern part of Mysore State. Climatic conditions prevailing in other areas may alter the time of flushing.

throughout the year. This is, of course, accompanied by the formation of fresh branches, and as these leaves and branches are closely crowded together, we get an appearance quite similar to what is known as Witches' Brooms. These Witches' Brooms are produced by certain parasitic fungi which stimulate the branches of the trees attacked to excessive growth and leaf formation. Their occurrence and the fungi causing them have been but little studied in India, but they undoubtedly do occur on a fairly large number of trees. This resemblance of the spiked growth to the growth of Witches' Brooms was first marked by McCarthy to whom we owe so much of our information with regard to this important disease.

Another striking feature of Spike, and one on which sufficient stress has not been laid, is the paler green of the leaves which show symptoms of the disease. In later stages this becomes very marked and in many cases the spike leaves take on a distinctly reddish colour.[1]

McCarthy notes, in his report, the appearance of what he calls "yellow leaf" in certain trees which came under his observation and which he ascribed, not to any disease, but to exposure to the sun and to the dense growth of lantana at the bases of the trees affected. He does not connect this phenomenon with Spike Disease in any way, and seems to consider it only a passing phase as he notes that he has never seen it in large trees and that the larger of the trees having yellow leaves seemed later to be taking on a greener colour.

Another phenomenon connected with this disease which has been noticed by both Barber and Butler in their investigations in the field, is the appearance of what is known as phyllody. Both these authors, as well as others who have studied the disease in the field, have noted the striking fact that a branch showing an advanced stage of the disease never bears flowers or fruit. In the few cases where flowers have been observed on a spiked portion of a tree, this has always been in an early stage of the disease, and the flowers show almost invariably, in place of the normal floral structures, a tuft of leaf-like bodies. This is well illustrated in Plate II, Fig. 1, where we have, on the one hand, a typical inflorescence from a heathy tree (Plate II, Fig. 1A), and, on the other, an inflorescence from a spiked tree showing the phenomenon

[1] This is due to the formation of anthocyan in the sub-epidermal cells of the leaf.

of phyllody (Plate II, Fig. 1C). Figure 1B shows a single normal flower and Fig. 1D a flower exhibiting phyllody. While trees attacked by Spike do not usually show normal flowers and fully developed fruits, this sometimes occurs on the portions of the tree which still retain their normal appearance.

If we go beneath the surface of the ground and examine the roots of a tree attacked by this disease, we find again conditions that are not normal, but before discussing this part of the subject it is necessary to touch briefly upon one of the most interesting features of the Sandal tree,—its root parasitism. Through the admirable researches of Dr. Barber[1] with the microscope, supplemented by the field information so painstakingly collected by him and by Forest Officers, notably Messrs. P. M. Lushington,[2] M. Rama Rao,[3] and C. C. Wilson,[4] our knowledge of this root parasitism is, on many sides, very full. We know, in the first place, that the sandal tree is by no means particular with regard to the hosts which it attacks, the number already observed exceeding one hundred. We know further that the host plants belong to the most varied orders. Dr. Barber, in his excellent papers, has taught us the way the haustoria are formed and the manner in which they penetrate the host roots. The accompanying photographs (Plates III and IV and Plate V, Fig. 2) show some of the most striking features in connection with them.

As Barber has already pointed out, one of the chief functions of these haustoria must be the taking up of crude sap from the vessels of the host roots. They function, therefore, in much the same way as root hairs which are scantily developed on sandal roots. Barber has also shown that, during penetration, a ferment is active in

[1] To Dr. Barber we owe much of our knowledge of the root parasitism of the sandal. The following papers by him should be consulted by those wishing information on this subject.

(1) The Study of Sandal Seedlings, Indian Forester, Vol. XXX, No. 1, 2, p. 545, 1904.

(2) The Haustoria of Sandal Roots, Indian Forester, Vol. XXXI, No. 4, p. 189, 1905.

(3) Studies in Root Parasitism. The Haustorium of Santalum Album, Memoirs of the Department of Agriculture in India, Botanical Series, Vol. I, No. 1, Parts 1 and 2.

[2] P. M. Lushington, Notes on Sandal, Indian Forester, Vol. XXX, No. 1, p. 13, 1904.

[3] M. Rama Rao, Notes on Sandal, Indian Forester, Vol. XXIX, 1903 and Vol. XXX, pp. 248, 357 and 397. Notes on Sandal (Germination and Growth of Sandal Seedlings), Indian Forest Records, Vol. II, Part III, p. 137, 1910. Host Plants of Sandal, Indian Forest Records, Vol. II, Part IV, p. 159, 1910. This paper includes a very full list of host plants and associates of the sandal tree. He lists 252 associates of which about 140 have been definitely shown to be host plants.

[4] C. C. Wilson, Sandalwood, Indian Forester, Vol. XLI, No. 8, p. 247, 1915.

dissolving the cell walls of the outer layers of the host root and that the solvent action combined with physical pressure brings about the penetration. He did not, however, attempt to isolate the ferment. During the process of penetration the products of this solvent action are, in all probability, utilized by the sandal but on this subject we have, as yet, little information.

Plate V Fig. 1, shows a transverse section of a haustorium on Lantana. The section has been stained with iodine, which brings out clearly the deposition of starch in the cortex of the haustorium. Barber[1] has noted this occurrence of starch, but does not hazard any suggestion as to the source of the materials from which it has been formed. He notes that, in later stages of development, the starch disappears, and states that it is probable that this store of starch is used up during the actual penetration. Our observations agree in the main with those of Barber, but in addition we have found in healthy and active haustoria granules of a starch-like body in the tracheids. These are no doubt similar to the amylodextrin granules described by Dr. (Miss) Benson in her paper on Root Parasitism in Exocarpus.[2] Heinricher[3] has also described similar granules which he found in the haustoria of Lathraea spp. As is the case with Exocarpus, no sieve tubes can be made out in the vascular strands of sandal haustoria.[4] Whether the carbohydrates found in the tracheids and the cortex of the haustoria originate solely in the leaves of the sandal and are carried from there to the roots and haustoria, or whether a certain amount in addition to that which must be absorbed as a result of haustorial action on the outer layers of the attacked roots is taken up by the haustoria from the host roots after penetration remains to be investigated. The whole subject of the function of haustoria is a most important one from the standpoint of the investigation of spike disease as well as from that of the normal development of the sandal tree. Plate V, Fig. 2, shows a haustorium which no longer exhibits any starch in the cortical tissue. This particular haustorium belonged to a spiked tree, but whether the disappearance of starch in this case is a

[1] Barber, The Haustoria of Sandal Roots, Indian Forester, Vol. XXXI, No. 4, 1905, p. 198.
[2] Benson, Root Parasitism in Exocarpus, Annals of Botany, Vol. 24, 1910, p. 667.
[3] Heinricher, Cohn's Beiträge z. Biologie d. Pfl., Bd. VII, p. 896, p. 342.
[4] This fact has already been noted by Barber in his work on Sandal Haustoria.

symptom of the disease or is simply due to the maturity of the haustorium was not established. The internal changes produced in haustoria as a result of the disease have still to be studied.

To return to the question of the changes brought about in sandal roots and haustoria by this disease, to Barber belongs the credit of first pointing out the fact that, in trees in an advanced stage of spike, the haustoria and the fine root ends are dead. The death of the haustoria and root ends seems, in fact, to keep pace with the progress of the disease. As the sandal tree is a root parasite and probably obtains most if not all the nutriment from the soil solution, indirectly through the roots of other trees by means of its haustoria, the death of these structures must naturally go hand in hand with the death of the sandal tree itself.

If we examine the internal structure of a diseased tree as compared with a healthy one, we find here again certain marked differences. Both Barber and Butler have noted most of them already, so it is not necessary for us to dwell upon this side of the subject. In the first place we have the marked deposition of starch in affected branches and leaves. Normal leaves show very little sign of starch deposition at any time. The normal twigs show an accumulation of starch only at the time of the year when the leaves are mature. At this time the quantity of starch in healthy twigs may be considerable. In spiked leaves, the starch is distributed throughout the parenchyma and is especially pronounced in the sheaths of the fibrovascular bundles. There is no marked difference to be noted microscopically with regard to the amount of starch in a spiked leaf at different times of the day and, as a matter of fact, it is necessary to shade such leaves for several days in order to make the starch disappear. Even then it persists in the fibrovascular sheaths. The starch grains found in the leaves are minute and are present several together in a chloroplast. As many as eight or nine such grains have been counted in one chloroplast. Plate VI gives two illustrations of a section of a diseased leaf showing the presence of starch grains and their relation to the chloroplasts.

In the twigs of spiked trees, the starch occurs in large quantities as grains of considerable size in the pith, medullary rays, and wood and bast parenchyma, whereas in the

normal twig such grains are rarely to be found, except as already stated at the time when the leaves are matured. This deposition of starch is to be found in twigs which, as yet, show no outward sign of the disease. Plate VII, Fig. 2, shows a cross section of a spiked twig with abundance of starch in the pith and the medullary rays. Plate VII, Fig. 1, shows a healthy twig in which there is no sign of starch.

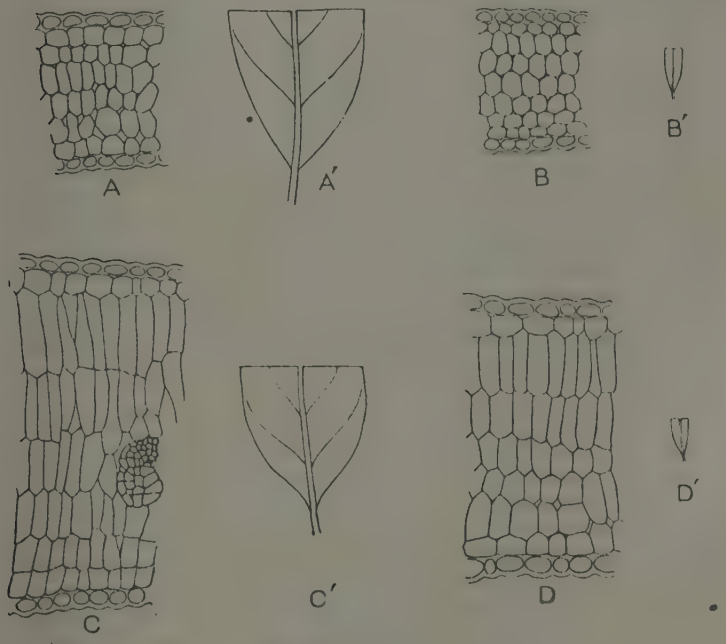
Other histological alterations noted by Butler are, (*a*) an increase of stomata per unit area in the case of spiked leaves, (*b*) change in position of the stomata relative to the surface, the stomata coming to lie flush with the latter, (*c*) hypertrophy of the mesophyll of spiked leaves leading to an increase of thickness, and (*d*) reduction in the formation of vascular tissue in the stem in advanced stages of the disease.

Our observations differ from Butler's in important points. In the first place, we have not been able to find any regular difference in the number of stomata per unit area in diseased and healthy leaves. This observation has been checked by counts made over equal areas and by drawings made to the same scale by means of the camera lucida. With regard to the position of the stomata, we find these just as much sunken below the surface in diseased as in healthy leaves. With regard to cellular hypertrophy in diseased leaves, again our observations do not agree with those of Butler. His description of the change of structure is as follows :—

> "The leaf is thickened in substance while much diminished in area, this hypertrophy being due not so much to an increase in the number of layers of cells as to their elongation at right angles to the surface of the leaf. This is especially so towards the upper surface of the leaf where the sub-epidermal cells are about twice as long as those of the healthy leaf; thus a well-marked palisade parenchyma is formed which is absent in the healthy leaf."

The accompanying text figure 2, A to D, shows cross sections of diseased and healthy leaves both young and old. As they are from drawings made with the camera lucida under exactly the same power, they are in every way comparable. They were also taken from the same relative positions in the various leaves. These drawings reveal the following facts. In young leaves, which have, however, reached their full dimensions as regards area, the cells of the mesophyll, in both diseased and healthy leaves, show

practically no elongation. The number of layers of cells is also approximately the same, six or seven layers of mesophyll cells being the rule. With the ageing of the leaves, these mesophyll cells elongate at right angles to the surfaces of the leaves, and this elongation is even more marked in healthy than in diseased leaves. The greatest elongation occurs in cell layers nearer the upper surface of the leaf in both cases. It may be noted here that Butler appears to have made his observations on material collected fairly early in the year at which time the



TEXT-FIGURE 2.

A, cross section of young healthy leaf; A′, outline of leaf from which section A was taken; B, cross section of young spiked leaf; B′, outline of leaf from which section B was taken; C, cross section of mature healthy leaf; C′, outline of leaf from which section C was taken; D, cross section of mature spiked leaf; D′, outline of leaf from which section D was taken.

great majority of healthy leaves are young. On the other hand, as one of the peculiarities of diseased trees is that growth continues throughout the year and as spiked leaves mature much more rapidly than do healthy ones, mature spiked leaves are to be obtained in abundance at any season. It seems highly probable, therefore, that Butler

really compared the structure of young healthy leaves with mature spiked ones which would, of course, give the results which he describes.

With regard to alterations noted by Butler in the formation of wood in twigs and stems, there are no doubt differences in this regard but they are by no means striking. We may say, in general that, as regards internal structure, the disease produces practically no alterations which can be made out by a histological examination, barring the one important feature so characteristic of the disease—the deposition of starch in the parenchyma of leaves and branches.

We see, then, that the histological differences between diseased and healthy trees are slight. What about the differences in chemical composition? Up to the present, work has been confined to a study of the chemical composition of the leaves. For this purpose, samples were collected during the latter part of 1915 and the earlier part of 1916 from a number of different areas where spike is prevalent. The analyses were confined chiefly to an estimation of the nitrogen content and of the ash constituents, though in one case an estimation of the amount of carbohydrate present was also made. The results of these analyses are given in Table 2. They are calculated on the dry weights of the samples.

These results show a strikingly greater percentage of nitrogen and a decidedly greater percentage of most of the ash constituents in the healthy leaves. This is especially marked in the case of lime where the percentage present in healthy leaves varies between somewhat over two times to almost eight times the amount found in spiked leaves. One might be tempted to consider that there was some causal connection between this poverty in necessary plant ingredients in spiked leaves, and a lack of these materials in the soil. It is true that the sandal tree gets most, if not all, of these materials by way of its host plants, but it is conceivable that these host plants may get along satisfactorily with food concentrations which are too low for the sandal. The analyses of soils from various sandal areas, however, do not lend any support to this view, as will be seen from Table 3. All of these samples were taken from areas where spike had put in its appearance. The figures given, indicate clearly that the occurrence of the disease does not depend upon the fertility of

TABLE 2.—ANALYSES OF SANDAL LEAVES.—RESULTS CALCULATED ON WATER-FREE MATERIAL.

Register No.	Description	N.	Loss on Ign.	Ash.	Ins. Res.	Fe_2O_3	P_2O_5	CaO	MgO	K_2O	Starch
1	2	3	4	5	6	7	8	9	10	11	12
1113	Healthy Sandal leaves (Bangalore) ...	2·67	84·17	15·83	1·08	·12	·59	5·42	·55	2·09	6·41
1115	Spiked do do ...	1·83	92·54	7·46	3·25	·08	·29	·72	·76	1·44	11·95
1572	Healthy leaves (Mysore)	4·88	88·28	11·72	·75	·08	·76	3·67	·78	2·23	...
1573	Spiked do do	2·18	93·28	6·72	·40	·09	·42	1·40	·50	2·00	...
1574	Healthy leaves (Heggaddevankote) ...	2·75	88·09	11·91	1·47	·06	·54	2·66	1·39	1·80	...
1575	Spiked do do ...	2·45	92·98	7·02	·86	·09	·48	1·23	·55	2·04	...
1576	Healthy leaves (Hunsur) ...	1·81	79·86	20·14	9·50	·18	·54	2·50	1·51	2·30	...
1577	Spiked do do ...	1·51	93·09	6·91	2·36	·13	·40	·64	·72	1·31	...

the soil. The first three samples, which are from diseased areas, are markedly richer in both nitrogen and lime than the last three which are from healthy areas. The amount of phosphoric acid and potash present also shows no relation to the presence or absence of the disease.. It is also clear that the lime magnesia ratio has nothing to do with the disease, as this varies very markedly in the different samples analysed. It appears clear, therefore, that there is little hope of checking or curing the disease by the application of fertilizers.

TABLE 3.—ANALYSES OF SOILS FROM SPIKED AND HEALTHY SANDAL AREAS.

Locality	N	P_2O_5	K_2O	CaO	MgO
1. Mysore, Chamundi Hillside ...	0·145	0·040	0·51	1·39	0·80
2. Hunsur	0·133	0·032	0·27	0·55	0·77
3. Heggaddevankote	0·082	0·032	0·25	0·34	0·59
4. Bangalore, Residency compound	0·049	0·027	0·41	0·11	0·06
5. Bangalore, Laboratory compound	0·045	0·072	0·23	0·14	0·10
6. Hebbal Farm	0·039	0·027	0·11	0·12	...

To return to the composition of spiked and healthy leaves, it is interesting to note that the total ash in healthy leaves is, in most cases, about twice as great as in spiked leaves while in one case it is three times as great. This, together with the great difference in lime already noted, points to the probability that spiked leaves are really comparable in composition with young healthy leaves. The results of investigations on the leaves of many plants have shown that, as the leaves become older, they tend to become richer in ash constituents and more especially in lime. The samples of both healthy and spiked leaves which were taken for analysis were, for the most part at least, fully mature, as is indicated by the time of the year at which they were taken (December and January); it remains, therefore, to ascertain by the analysis of young healthy leaves whether they pass through a stage which is comparable in chemical composition to that of the spiked leaves. If this proves to be the case, we shall probably be justified in concluding that, in spike, the leaves are for some reason checked in an early stage of

their development. This may be due, as Barber has supposed, to the cutting off of the supply of soil salts owing to the gradual death of the end roots and the haustoria. It must, however, again be pointed out that, as far as anatomical structure is concerned, fully developed spiked leaves approximate somewhat closely to fully developed healthy ones. Enough has been said to indicate that there is still much to be done before we shall have cleared up the question of the chemical differences in the leaves of healthy and diseased trees, while the question of alterations of chemical composition in other organs still remains practically untouched.

Up to the present we have been discussing the symptoms of the disease. The question of its cause has not been touched upon, but before taking up this phase of the subject, it may be well to touch briefly upon somewhat similar diseases of other plants in India which have been brought to light. We owe much of our information with regard to these diseases to the various Forest Officers who have interested themselves in the investigation of Spike Disease, more especially to Messrs. P. M. Lushington,[1] M. Rama Rao[2] and H. Srinivasa Rao.[3] The plants which have been reported as showing symptoms similar to Spike Disease of sandal are the following:—

Zizyphus œnoplia, *Dodonæa viscosa*, *Pterolobium indicum*, *Argyreia cuneata*, *Solanum indicum*, *Cassia auriculata and Ficus tsiela.*

Of these plants we have examined microscopically, only *Zizyphus œnoplia* and *Argyreia cuneata*. The symptoms in *Zizyphus* are almost identical with those of sandal spike. The leaves are much reduced in size and distinctly paler in colour, so much so, in some cases, as to be quite etiolated either wholly or in part. The internodes are very much shorter so as to give the spiked growth a bushy appearance. An internal examination shows a great accumulation of starch in the parenchyma of leaves and twigs and an examination of the roots shows the fine root ends dead. No fungus growth could be made out in an examination of any part of the diseased plants. Plate VIII shows the change produced in *Zizyphus* by the disease. A shows a healthy branch bearing normal leaves.

[1] P. M. Lushington, Note on Spike Disease of Sandal, Indian Forester, Vol. XLII, 1916, p. 65.
[2] Referred to by Mr. Lushington in the paper noted above.
[3] H. Srinivasa Rao, Mysore Forest Selections, 1906, p. 46.

B shows a portion of a spiked branch revealing the external symptoms above described.

Argyreia cuneata, on the other hand, appears to exhibit only one of these symptoms, *viz.*, the reduction in the size of the leaves. A microscopic examination reveals no deposition of starch in leaves or twigs. Instead, a very marked growth of fungus mycelium was found in the vessels of the twigs and it seems probable that the disease is, in this case, caused by a fungus. Probably a similar fungus cause will be found in the case of at least some of the other diseases which have been supposed to resemble Sandal Spike.

In addition to the plants above mentioned, symptoms similar to Spike have been found by us in a number of other species during the progress of these investigations. These species are *Tephrosia purpurea*, *Crotolaria retusa*, *Vinca rosea*, *Stachytarpheta indica* and *Datura stramonium*. Plates IX and X show the appearance of diseased specimens of some of these species. In all of these five species we found internal symptoms similar to those described for *Zizyphus œnoplia* as regards deposition of starch. The root systems of some of these plants have not been examined, so we are unable to say whether the symptoms extend to that portion of the plants as well. In the case of *Stachytarpheta indica*, the phenomenon of phyllody is particularly common as a symptom of the disease.

The fact that many of these plants are common in areas where sandal grows, lends a special interest to the study of their diseased condition, and the question whether the markedly similar symptoms occurring in so many species belonging to different orders are all produced by the same cause is one of the greatest importance. The fact that the plants above mentioned are not root parasites will allow pot culture experiments with them to be carried out much more easily than they can be in the case of sandal. A large number of pot and water culture experiments have, as a matter of fact, been attempted with sandal, but they have yielded very little information, largely because of the difficulty in getting sandal to grow normally under these conditions. These experiments are therefore not dealt with in this paper. A series of experiments, similar to those tried on sandal, to test the effect of various different soil conditions and of the presence or absence of various different nutrient salts, will be started shortly, using

Zizyphus œnoplia and others of the plants named above as material. This should furnish us with a great deal of valuable information which can be applied in a further study of Sandal Spike itself.

Various theories have been advanced with regard to the cause of Spike disease, most of which have had to be abandoned as untenable with the gradual increase of information on the subject. In any consideration of the origin of a disease, it is necessary to differentiate carefully between primary causes and predisposing factors, something which has not always been done in the various communications on this disease. Predisposing factors are those which tend to weaken the plant or in some way to make it more susceptible to a disease, but if the primary cause is absent in any locality, no combination of predisposing factors is able to produce the disease. To make this clear, a concrete example may be taken. Koleroga of the Areca Palm, commonly occurring in the Mysore Malnad, is a disease produced by a definite fungus which is the primary cause. The main predisposing factor is a moist condition of the atmosphere resulting from the heavy rains of the south-west monsoon. If, however, the fungus causing the disease is not present in an areca nut garden, the disease will not appear, no matter how great the rainfall or how saturated the air is with moisture. On the other hand, if this contributory factor of excessive moisture is not present, the fungus can make no headway and the disease either does not appear, or, at the worst, does not spread in a serious manner.

In connection with the study of Spike Disease, a large number of external factors have at different times been cited as its cause. Among these may be mentioned, the occurrence of fires, the presence of lantana, overgrowth by climbers, lack of vigour in the vegetation associated with sandal and unsuitable soil conditions. Most of these factors might, no doubt, contribute to the spread of a disease in that they might decrease the vitality of the sandal and so make it more susceptible to attack. It is conceivable that one or more of them might be the cause of the disease but it would be extremely unsafe to come to that conclusion simply because the disease appears only or mainly where such factors come into play. It is necessary to consider briefly the various external factors mentioned in order to ascertain whether there are

sufficient grounds for considering any of them as primary causes of, or contributing factors to, the disease.

The action of fire as a factor in connection with the appearance and spread of this disease was first noted by McCarthy[1] in the Memorandum cited above. The following quotation gives the results of his observations on the subject:—

"As regards the first appearance and progress of the disease, there are two very different ways in which what looks like the same kind of Spike appears upon the trees, and this it is which seems to make it doubtful that the thing is a disease and not merely an after effect from fire. I will proceed to describe. It is a very common sight to find medium and large-sized trees which have been recently rather severely burnt but not killed, and even main branches of such trees, the other portions of which have escaped, which have replaced the burnt-off foliage by a numerous, broomlike, growth of fine shoots springing up all round the main stem and larger branches and presenting the characteristic of Spike above described. Sometimes the new shoots are so many and thick that the trunk at a distance looks as if it was clothed with a kind of moss although the shoots are really from four to eight inches long. These are the cases of worst damage and then the stiff branches jut out of the new foliage in a stag-headed manner. This appearance of epicormic branches is, of course, not peculiar to the Sandal tree. *Terminalia tomentosa* frequently shows the same thing after severe burning. At other times when the fire has passed under the tree and has not been fierce enough to strip it entirely, only the lower branches show the symptoms; whilst the upper, for that growing season at least, and perhaps the next, retain their natural foliage. Again, trees under which a small fire has been known to pass last season, although showing no sign of damage in the trunk or foliage, break out all over with Spike the following growing season. But in all these cases the disease is bound, in course of time, to kill the tree. In the above class of cases, cause and effect immediately succeed one another and are very apparent.

"In the cases now to be described, an apparently identical Spike appears in an exactly opposite manner; and this appearance is far more common than the other. Here a well-grown healthy tree of sometimes as much as twenty-one inches girth, around which there are no indications of recent fire, although the situation is almost invariably one which may have been burnt over a few years back, shows at the tip of one branch one or two diseased shoots. Next year there are more such shoots, and so on until half the branches are affected. Then still on until there is perhaps only a single branchlet left bearing natural shoots, flowers and fruit. Finally, the spike covers the whole tree but how long it takes to do this I have not been able to ascertain yet. I believe the disease runs its course in from three to five years."

Lushington[2] has also expressed the view that the

[1] McCarthy loc. cit., p. 17.

[2] P. M. Lushington, Office note, dated 30th Oct. 1903, to Conservator of Forests, S. C. Madras. Published in Mysore Forest Selections, p. 36, 1906.

disease will eventually be traced to fire, but adds that if it be due to this cause, it is difficult to understand why it has appeared so recently. Butler[1] looks upon fire as a predisposing cause, but does not consider it the primary one. Muthannah,[2] who did not look upon 'spike' as a specific disease, considers fire to be one of the causes.

If we sum up the evidence available, and take into account our own observations, we must conclude that while fire may be a contributing factor, hastening the spread of the disease, it cannot be considered as its cause. There is not the slightest doubt that the disease has appeared in areas which have been carefully protected from fire and where fire has not occurred for many years previous to the appearance of the disease. On the other hand, there are very many instances where the occurrence of fire has not been followed by any outbreak of the disease.

The presence of lantana has also been looked upon as a factor contributing to the disease. McCarthy, in the first place, suggested that lantana had an indirect effect in that the presence of lantana thickets increased the frequency and severity of fires, and since then it has been suggested by various authors[3] that this plant might have a more direct effect either by depriving sandal of something necessary to its growth or by communicating some new and unsuitable substance to the sandal. Since these observations were made, however, it has been clearly established that Spike disease occurs in areas where the connection of the attacked trees with lantana or an influence of lantana on the sandal trees attacked is out of the question.

With regard to soil and climatic conditions, these have been supposed to act in various different ways. In the first place, Muthannah, as already noted, believed that Spike disease was definitely restricted to areas where the soil is poor or contains a large percentage of kanker. This view is no longer tenable, and the analyses of soils from attacked areas given above show pretty clearly that the disease cannot be considered as due to any lack of essential plant foods in the soil. This officer also states that

[1] Butler, loc. cit., p. 8.

[2] Muthannah, Government of Mysore, Forest Proceedings, 3rd July, 1903, p. 23.

[3] (1) *Vide* observations on Spike Disease by Mr. H. C. Hill, Late Inspector General of Forests to the Government of India, quoted in Mysore Forest Selections, 1906, p. 26; (2) Letter from Dr. Butler to Mr. Muthannah, published in same selection, p. 26; (3) Barber, loc. cit., Indian Forester, Vol. XXIX, p. 28.

water-logged soils are inimical to the growth of sandal but does not definitely express the view that this is to be considered as one of the causes of the disease. There is abundance of evidence to show that waterlogging, with the resultant depression of aëration in the soil, cannot possibly explain the appearance of the disease, as some of the areas attacked are exceptionally well drained. On the other hand, Whitehead[1] has recently sought for the cause of the disease in an exactly opposite soil condition, a reduction of the supply of water in the soil. Unfortunately, however, his conclusions seem to be based on purely theoretical considerations, and are not the result of personal observation. Mr. Tireman has kindly furnished me with information with regard to rainfall in infected areas in Coorg, which shows that the disease is prevalent over areas with annual rainfalls varying between 70 inches and 40 inches. It is hard to believe that, in an area having a seventy-inch rainfall, the water supply in the soil would be less adequate than in parts of Mysore State which have an annual rainfall of less than thirty inches and where Spike has not yet appeared. Mr. Tireman also informs me that, during June and July, 1916, the disease played great havoc among some very fine trees growing on the bank of the Cauvery near Fraserpet, and remarks that these trees could not possibly ever be short of moisture as the river never dries up. Whitehead also suggests that the disease may be the result of the overstocking of sandal; but this view hardly seems tenable as many very well stocked areas have remained free from the disease while others much more poorly stocked have suffered severely.

The above discussion practically covers all the theories which have been advanced to explain the appearance and spread of the disease as a result of external factors. The facts and considerations presented show, I think, quite clearly that the search for an immediate cause among the various external factors enumerated has not led us very far towards a better understanding of the disease. On the other hand, it seems highly probable that, once given the primary cause, whatever it may be, in any particular area, several of the factors mentioned may play an important *rôle* in determining the virulence and rapidity of spread of the disease.

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[1] Whitehead, A Possible Cause of "Spike" in Sandal, Indian Forester, Vol. XLII, 1916, p. 243.

If we now turn to a consideration of other possible causes of the disease, we are naturally led to the question of an attack by some living organism, insect, bacterial or fungus. As a matter of fact, the possibility of a living cause for the disease was one of the first things which presented itself to investigators of the disease, and McCarthy,[1] in his original memorandum, notes that he could find no sign of fungus mycelium or fruiting bodies on the branches or leaves of attacked trees. Butler[2] also made a careful examination with results which he describes as follows:—

"A detailed microscopical examination was made of the tissues from leaf to root and checked by a series of preparations kindly lent to me by Mr. Barber. The first fact noted was the absence of any trace of parasitic action sufficient to account for the disease, in any part of the diseased trees. A few fungus hyphæ were found on epidermis and cortex, a few bacteria in the tissues and some probably saprophytic organisms on the roots; but these were similarly found in healthy trees and were, in any case, totally inadequate to produce such marked symptoms of disease."

Barber,[3] who, as will be seen later, is inclined to view the disease as primarily one of the roots, gives results of his microscopic examination practically identical with Butler on this point, while our examination has also revealed no fungus or bacterium which could be looked upon as the cause of the disease.

The possibility of insect cause has also not been lost sight of. The insect pests of sandal are few and unimportant. Stebbing[4] made a preliminary study of the borers attacking sandal, and gives three as more or less common *viz.*, the common red borer of coffee, *Zeuzera coffeæ*, *Nietner*, a longicorn beetle and a beetle belonging to the family *Siricidæ*. None of these can be looked upon as, in any way, connected with spike disease. With regard to insects attacking the leaves and twigs, there are a number of sucking insects, belonging to the families *Coccidæ* and *Jassidæ*, while there are occasionally signs of insects eating the leaves; but as no evidence has yet come to our attention that any of these insects occur in larger numbers in attacked areas than elsewhere, they can hardly be looked upon as probable causes of the disease.

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1 Loc. cit., p. 17.
2 Butler, loc. cit., p. 3.
3 Barber, Indian Forester, Vol. XXIX, 1913, p. 29.
4 Stebbing, Note on the Sandalwood Boring Insects of Madras, Indian Forester Vol. XXIX, 1903, Appendix.

Before discussing the results of our investigations of the cause of the disease, it is necessary to consider more fully the views advanced by Barber and Butler on the subject. As already indicated, Barber looks upon the disease as originating in the underground portion of the tree and bases his views upon his observations that, invariably in advanced cases of the disease, the root ends and the haustoria are dead. He pictures the origin and progress of the disease as follows:—

"An examination of the roots of spiked trees shows that their sucking organs, or 'haustoria' as they are technically termed, are absent or dead. It is a matter of some difficulty to follow the roots to their termination without breaking them. For two whole days my staff were carefully dissecting the earth away and laying bare the root systems of spiked and healthy trees, with the invariable results that all the root ends of the spiked trees were dead and rotten. That there is a root disease present, then, I have no manner of doubt. As regards the exact cause, I have not had the means of judging.

"Bearing these facts in mind, the following is what appears to happen in spiked trees, although I cannot profess that all the phenomena are explained. An injury occurs to the roots and their ends die; the supplies of salts and water are thus cut off from the plant. This does not take place all at once, for there are a great number of root-ends to be affected, and there is also a certain quantity of salts and water stored up in the stem and main roots; the shortage in the supply of the salts needful for seed formation accounts for the cessation of flowering; fresh roots are needed, and fresh leaves are called upon at an unseasonable period to produce the necessary material; the branches continue in their growth which is not terminated by the formation of a flower and new leaves are formed in rapid succession; the old leaves are thrown off and the new ones formed are smaller and smaller; by their active metabolism, the tissues, even to the smallest twig are filled with starch, which has no chance of getting away; the tree finally cannot stand the strain, and having used up all its supplies of salts and water, finally dies of exhaustion."

The above explanations can hardly be called satisfactory. In the first place, Barber is vague with regard to the injuries to the roots which are supposed to start the disease going. If these were mechanical in nature, it is very hard to understand how the injury to a few roots (mechanical injury of a large proportion of the roots of a tree seems out of the question) could lead to the death of other roots and haustoria far removed from the place of injury. There is no evidence that insect attack can be held responsible for root injury, so they cannot be called in to explain the phenomena, and, as already noted above, fungus or bacterial attack seems also to be excluded.

Undoubtedly, the root ends and haustoria die, but it seems *a priori* just as likely that their death is one of the later symptoms of the disease as that their death sets up the train of events in the above-ground portion of the tree which Barber has so well described. That the cutting off of salts and water might lead to a reduction of the size of the leaves is highly probable and, in fact, we have evidence that this actually does occur. Thus when sandal seedlings are transplated, they almost invariably put out smaller leaves than do seedlings whose root systems have not been disturbed, and this may very probably be due to the slowness in establishing haustorial connections with fresh host plants. There is, however, no evidence in such cases of an increased deposition of starch or of a more rapid formation of leaves accompanied by a shortening of the internodes which are such characteristic features of spike. The appearance of phyllody and the cessation or delay in flowering, both of which are usually supposed to be associated with excessive nutrition, can hardly be explained by a diminution of the supply of soil salts. There is, in fact, no evidence to show that an injury to the roots, however severe, can, of itself, call forth any more than the one symptom of Spike already noted (the diminution in size of leaves,) and even this symptom is never very marked. We must conclude, therefore, that there is no satisfactory evidence that spike is primarily a root disease, while facts which will be given later in this report go to show quite clearly that the disease can originate in the above-ground portions of the tree.

Butler's views on the cause and progress of the disease are important. He looks upon the disease as a nutritional one characterised by a forced carbon assimilation, and points out that a number of the symptoms typical of Spike disease are such as have been produced in other plants by exposing them to air containing an excessive quantity of carbon dioxide.[1]

His views as to the excessive carbon assimilation are as follows:—

"We have, unfortunately, as yet nothing to guide us as to the cause of the intensified carbon assimilation in spiked sandal. It would

[1] *Vide* Brown and Escombe. The Influence of Varying Amounts of Carbon Dioxide on the Photosynthetic Processes of Leaves and on the Mode of Growth of Plants, Proc. Roy. Soc., B. Vol. 70, 1902, p. 397; also Farmer and Chandler, On the Influence of an Excess of Carbon Dioxide in the Air on the Form and Internal Structure of Plants, Proc. Roy. Soc., B. Vol. 70, 1902, p. 413.

be absurd to suppose it due to unusual quantities of carbon dioxide in the atmosphere. The effects of such a condition would have been rapidly visible on the human population of the country affected. The cause must, therefore, be sought in the plant itself. For some reason or other, the tree or some isolated branches of it, suddenly proceeds to manufacture starch out of all proportion to the demand for this food by the tissues. As a result of this, growth is forced, the tree gets no rest, and dies of exhaustion, the result of starch poisoning.

"In the plant itself a disease of the stomata, or of the starch-forming cells, or the circulation of some substance in the sap, might each account for the forced production of starch. The stomata are certainly much altered, lying flush with the surface of the leaf instead of forming a depression in the epidermis, as in the healthy plant. But I can find no apparent cause for this, and the condition is probably secondary to the leaf hypertrophy. The starch-forming cells are apparently healthy. The existence of a poison in the sap can only be determined by a series of infection experiments carefully conducted over a number of years. I fail to see how the mere destruction of root-ends could possibly result in an increased formation of starch, for that starch is formed in excessive quantity and not merely stored up because not consumed in normal amount as fast as it is formed, is evident from the fact that growth is actually increased and that, therefore, more starch than usual must be actually consumed. But the destruction of rootlets may well be an early evidence of the action of some poison circulating in the plant. That this poison, if present, actually enters through the root-ends, we have nothing to show, since no organism has as yet been found in the roots that would be likely to be a source of disease. The fact that in addition to the free rootlets, the organs of attachment to other roots, or 'haustoria,' through which a portion of the food of the sandal tree is absorbed are also dead in spiked trees tells against a local disease of the rootlets alone. The existence of a poison circulating in the sap seems, therefore, to be the most probable explanation of the suddenly intensified starch manufacture."

The view that the disease is due to a poison or ferment circulating in the sap was suggested originally by McCarthy,[1] as will be seen from the following quotation :—

"The conclusion that appears forced upon one is that there is no fungal attack, but that something of the nature of a ferment is communicated to the sap, perhaps originated by contact with or proximity to the roots or foliage of burning lantana."

While we have not been able to confirm some of Butler's observations, notably with regard to the hypertrophy of the leaf, the increase in the number of stomata and the change in their position relative to the surface of the leaf, there is almost certainly a decided increase in the activity of growth. No quantitative measurement of the growth in diseased and healthy trees within a given time has, however, yet been made. It is conceivable that,

[1] Loc. cit., p. 19.

although the number of leaves and twigs formed within a given period is very much greater in a diseased than in a healthy tree, this might be counterbalanced by the greater amount of tissue formed in each leaf and twig in the case of the healthy trees. Although in healthy trees fresh leaves and twigs are normally developed only during definite seasons of the year, there is a continuous growth in thickness which certainly exceeds the growth in corresponding organs in diseased trees. This question requires further investigation, but there, nevertheless, seems to be little doubt that the total growth on a diseased branch in the early stages of the disease is greater than that on a corresponding healthy branch.

The question of the deposition of starch in spiked leaves and twigs is one that requires careful study. We have scarcely more than begun the investigation of this important subject, but certain of the preliminary results obtained seem worthy of publication. We do not know definitely what are the first products formed by the combination of carbon dioxide and water in leaves although a great deal of work has been done on the subject by investigators in all parts of the world. We do know, however, that the formation of starch is a secondary phenomenon. The products of carbon assimilation are transported from the leaves in the form of soluble carbohydrates such as sugars (maltose, etc.), and if, for any reason, the concentration of sugar in the sap of the leaf cells becomes too great, starch, an insoluble carbohydrate, is formed and deposited. In order that this may be carried to another part of the plant to be used in building up tissue or in supporting any other life process, it must be changed back into a soluble carbohydrate. For this, the presence of a special dissolving ferment, diastase, is necessary. To Brown and Morris[1] we owe much of our knowledge of the presence and action of leaf diastase, and their methods have been largely followed in the present investigation.

In spiked leaves and twigs we have a large deposit of starch and, as already stated, Butler associates this with an excessive carbon assimilation in the diseased leaves. It seemed to us necessary, however, to form an estimate

[1] It would be out of place in a report like this to discuss the immense literature dealing with the formation and translocation of carbohydrates in the leaf. The subject is dealt with in the classical work of Brown and Morris, The Chemistry and Physiology of Foliage Leaves, Journal of the Chemistry Transactions, Vol. LXIII, 1893 p. 604, where the older literature on the subject is discussed. See also Czapek, Biochemie der Pflanzen, Vol. I, p. 382, 1905.

of the relative diastatic activity in diseased and healthy leaves as it seemed possible that the deposition of starch might be explained to some extent at least by a disturbance in translocation. If carbon assimilation is greatly increased, one might expect to find a corresponding increase of diastatic activity to deal with the excess of the products formed.

In order to ascertain whether there is any difference in the actual translocation of carbohydrate in the two cases, the more accurate method would be, following the work of Brown and Morris, to estimate the difference in amount of starch per unit area in leaves removed at the close of the day as compared with those removed after remaining all night on the tree. The carbon assimilation in green leaves takes place normally only in the presence of sunlight. The result is that the formation of carbohydrates from carbon dioxide and water ceases in the evening. On the other hand, translocation, or the carrying away of carbohydrates from the leaves to other parts of the tree, takes place throughout the twenty-four hours. In the day time, this translocation is in many cases masked by the assimilation, but at night it becomes apparent, with the result that in healthy leaves much if not all of the starch which is deposited in the leaves during the day is redissolved and carried off. The difference between the amount of starch found in leaves at the close of the day and that found in similar leaves at the end of the night would give a fairly accurate measure of the amount translocated during the night.

Unfortunately, the small size of spiked leaves makes such a direct estimation difficult if not impossible. It was thought more advisable therefore to attempt to obtain an estimate of diastatic activity by testing the action of a weighed quantity of leaf powder on soluble starch, following the method of Brown and Morris[1] for the purpose.

The experiments were conducted as follows:—Leaves from healthy and diseased trees were plucked at the same time. The leaves were then split into right and left halves along the midrib and thoroughly dried, right and left halves being kept separate. The dried leaves consisted of four separate lots, *viz.*, spiked leaves, right and left halves, and the same for the healthy leaves. The separate lots were then ground to powder and two equal

[1] Loc. cit., p. 637.

samples (·5 grms.) from each lot were added respectively to two tubes containing 50 cc. of 2% soluble starch solution; 5 cc. of chloroform to 1 litre of solution was added to prevent bacterial action. These tubes were then incubated at approximately 50° centigrade for 48 hours and at the end of that time an estimation was made of the amount of sugar formed in each tube. A similar series was prepared, boiled to destroy the diastase and used as a check. The results are given in the accompanying table. Estimations were made by the ordinary Fehling's method.

TABLE 4.—DIASTATIC ACTIVITY OF DISEASED AND HEALTHY SANDAL LEAVES.

Description—Untreated leaves	Weight of maltose formed in grammes per 50 cc. soluble starch solution	Average for two halves of leaves	Average for whole leaf
Healthy leaves, right side ...	·168	·171	
Do	·174		·179
Healthy leaves, left side ...	·186	·186	
Do	·186		
Spiked leaves, right side ...	·096	·096	
Do	·096		·098
Spiked leaves, left side ...	·096	·099	
Do	·102		

The results for the boiled solution, in which no diastatic action could take place, were so low in every case as to be negligible, and are not included, as they do not in any way affect the relative values given in the above table. The difference in diastatic activity is very striking, that of the healthy leaves being almost double that of the spiked leaves. As will be noted, only one product of diastatic activity, maltose, has been estimated but as, under otherwise similar conditions, the relation between the various products of starch decomposition would be the same, this gives us, with sufficient accuracy, the relative diastatic action in the two cases.

This experiment was repeated on a much more elaborate scale, samples being collected every three hours throughout the twenty-four. The object of this was to

trace the changes in diastatic activity in both diseased and healthy leaves throughout a complete day. The results are given in the following table, but here averages for the whole leaf are given.

TABLE 5.—DIASTATIC ACTIVITY OF DISEASED AND HEALTHY SANDAL LEAVES.

Time	Description	Weight of maltose formed in grammes per 50 cc. of 2% soluble starch solution	
12 P.M. ...	Healthy leaves ...	·206	
	Spiked leaves ...	·072	
			Average for 24 hours
3 A.M. ...	Healthy leaves ...	·186	Healthy leaves ... ·176
	Spiked leaves ...	·114	Spiked leaves ... ·136
6 A.M. ...	Healthy leaves ...	·186	
	Spiked leaves ...	·186	
9 A.M. ...	Healthy leaves ...	·156	
	Spiked leaves ...	·114	
12 NOON ...	Healthy leaves ...	·174	
	Spiked leaves ...	·111	
3 P.M. ...	Healthy leaves ...	·150	
	Spiked leaves ...	·150	
6 P.M. ...	Healthy leaves ...	·150	
	Spiked leaves ...	·174	
9 P.M. ...	Healthy leaves ...	·198	
	Spiked leaves ...	·165	

Brown and Morris, in giving the results of their experiments on diastatic activity, show them as the amount of starch hydrolised by 10 grammes of dried leaf. To compare the figures given above with those given by these authors, it is, therefore, necessary to multiply our figures by 20. If this is done we find the figures are very similar to those obtained by Brown and Morris for the leaves of a large number of the plants with which they experimented. It may be noted here that, in the experiments of these authors, they kept the temperature at 30° centigrade. The higher temperature of 50° centigrade was chosen in our experiments because it was found that it increased the vigour of diastatic action.[1]

[1] *Vide* also Green, The Soluble Ferments and Fermentation, p. 66, 1901, where the optimum temperature for translocation diastase is given as varying from 45°-50 centigrade.

A consideration of the figures given in the above tables brings out a number of interesting points. In the first place, we see that the diastatic action of spiked leaves is distinctly lower than that of healthy ones. In the first experiment, where the leaves were collected at only one time of the day (9 A.M.) the healthy leaves showed almost double the diastatic activity of the spiked ones. In the second experiment, where the leaves were collected at intervals throughout day and night, the difference is not so great. Even here, however, healthy leaves displayed, on an average, about 30 per cent greater diastatic activity than spiked ones. The spiked leaves used in these experiments were those showing an early stage of the disease and were taken from trees which were only partially spiked, so the comparison is quite a fair one.

Another interesting point which is revealed by a consideration of the figures given in the second table, is the difference in diastatic activity in both diseased and healthy leaves at different times of the day and night. Brown and Morris, in their investigations, established that there is a periodic variation in the diastatic activity of leaves. The following table taken from their paper shows this clearly:—

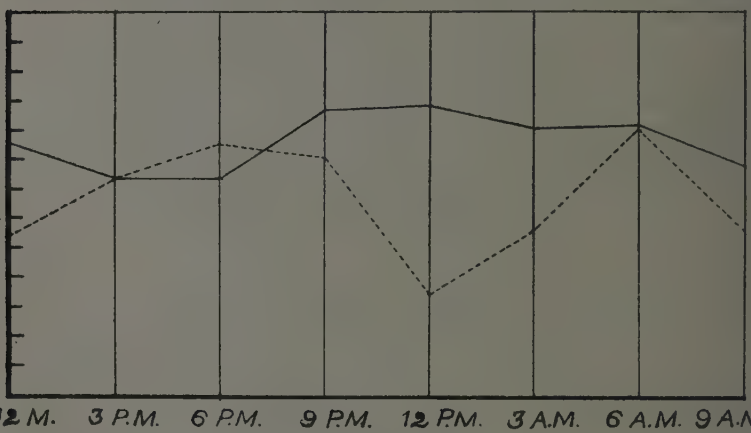
TABLE 6.—EXPERIMENTS WITH TROPÆOLUM MAJUS.

——	Diastatic activity	Increase of diastase per cent
1 Sept., 10 Half-leaves plucked at 5 P.M.	1·963	...
Do 10 P.M.	2·663	35·6
2 Sept., 22 Half-leaves plucked at 3 P.M.	1·100	...
Do 11 P.M.	1·874	70·3
3 Sept., 28 and 29 Half-leaves plucked at 4-15 P.M.	1·006	...
Do do at 5-30 A.M.	1·645	63·5

These results indicate an increase in diastatic activity during the night, and if we examine the figures showing the diastatic activity of healthy sandal leaves, we find a similar state of affairs, the lowest diastatic activity occurring in the afternoon at 3 and 6 P.M. and the highest in the middle of the night. If we examine the diastatic activity of diseased leaves, on the other hand, a very different state of affairs is found. Here the lowest diastatic

activity occurs at midnight, the highest at 6 A.M. with a second rise at 6 P.M. The differences are well brought out in the accompanying chart (Text-fig. 3), in which the variation in diastatic activity of the leaves both healthy and diseased for the twenty-four hours are shown graphically.

We do not propose to discuss the possible reasons for the periodicity in diastatic activity. The matter has been discussed by Brown and Morris[1] without their coming to any very definite conclusions. It is sufficient here to point out that the phenomena exhibited by the diseased leaves indicates, not only a reduction in the total diastatic activity but also a disturbance in its normal periodic



TEXT-FIGURE 3.

Chart showing variation in diastatic activity in healthy and spiked sandal leaves. The solid line represents variations in the healthy leaves: the broken line those in the diseased leaves. The ordinates represent ·02 grammes maltose: the abscissæ periods of 3 hours.

variation. The question of diastatic action in the twigs and in other parts of the tree is also important, but there has been, as yet, no opportunity to take it up. A thorough discussion of the whole question will be possible only after the results of much more detailed experimental work are available.

If we turn now to a consideration of the rate of carbon assimilation in diseased and healthy leaves, we are faced with a more difficult question. A number of different

[1] Loc. cit., pp. 647-649.

methods of estimating carbon assimilation have been used at various times and some of these have given quite satisfactory results. The method of Sachs[1] which was subsequently found by Brown and Morris[2] to give, with proper care, satisfactory results could not be used because of the small size of leaves, especially in the case of diseased ones. The diastase method also used by Brown and Morris[3] did not yield satisfactory results; so we turned to an indirect method which has been developed by Brown and Escombe[4] and by Blackman.[5] By this method, the carbon assimilation in leaves is estimated by measuring the amount of carbon dioxide taken up from the air per unit area of leaf. The method gives only relative and approximate figures, as it does not take into account the amount of carbon dioxide formed in the leaf in the process of respiration. Relative values are, however, all that are required, as the object is not to ascertain the total quantity assimilated per unit area by healthy and diseased leaves but to find out whether important differences exist. The difficulties in obtaining apparatus from abroad have compelled us to construct our own and this has entailed great delay. The results of the experiments are, therefore, not yet available and will be published in a subsequent paper. It seems, however, highly probable that we shall by this method be able to discover early symptoms of the disease which cannot be made out by a microscopic examination. In other words, it seems likely that before there has been any marked deposition of starch in leaf or twig, and long before there is any outward sign of the disease in the diminution of leaf area, an increase in the carbon assimilation will be found to take place. With the accumulation of starch in leaf and twig we may expect to find the rate of carbon assimilation gradually decrease till in the final stages of the disease it comes to zero.

The probable effect of the accumulation of starch in the leaves and twigs in decreasing assimilation appears to have been overlooked by both Butler and Barber. It is

[1] Sachs, Ein Beitrag zur Kenntnis der Ernährungstätigkeit der Blätter, Arbeiten des Botanischen Instituts in Würzburg, 3, 1884. The method consists in weighing definite areas cut out from leaves by a template before and after exposure to sunlight. The difference in weight was taken by Sachs to represent the increase due to carbon assimilation.

[2] Loc. cit., pp. 624-628. [3] Loc. cit., p. 628.

[4] Brown and Escombe, Researches on some Physiological Processes of Green Leaves, Proc. Roy. Soc., B, 76, p. 29, 1905.

[5] Blackman, On Vegetative Assimilation and Respiration. Phil. Trans. Roy. Soc., B, 186, p. 485, 1895.

interesting to note, in this connection that, as long ago as 1890, Saposchnikoff,[1] as a result of his investigations, reached the conclusion that "the accumulation of carbohydrates in leaves hinders the further production of those bodies, and, conversely, the quicker the carbohydrates are carried away from the leaf the better the leaf functions as regards assimilation."

As already noted, Butler inclines to the view that the disease is caused by a poison or virus which is communicable from one tree to another. He further considers that the rapid spread of the disease, in its early stages, points to the spread of the disease through the air while the later progress would indicate a spread through the ground from root to root. Butler compares the disease to "Peach Yellows," a serious disease of the peach tree in America, which, although no causative parasitic organism has been found, has been clearly proved to be infectious. It is, in fact, one of a steadily growing class of plant diseases which are undoubtedly infectious but a cause for which has not been definitely established. These diseases will be considered later in this report, as the work done upon them has considerable bearing upon the question of Spike Disease.

As we have seen, the most varied views have been expressed as to the cause of this disease. To some it is simply an expression of unfavourable external conditions and cannot be looked upon as communicable in any degree. To others its communicability has seemed quite certain. As a matter of fact, however, no definite pronouncement on this subject could be made until the results of definite experiments were obtained.

Butler, in 1904, following the work of Smith[2] on "Peach Yellows," attempted to produce the disease by budding. For the purpose, he took buds from diseased trees and grafted them on to healthy ones. Although he tried some one hundred and fifty budding experiments, none of the buds grew, so that the results of the experiments gave no information of value. In 1914, similar experiments were commenced by us in Bangalore, but it was soon found that budding was not a suitable method of grafting

[1] Saposchnikoff, Bildung and Wanderung der Kohlenhydrate in den Laubblättern, Berichte der deutschen botanischen Gesellschaft, 8, 1890, p. 233, cited from Brown and Morris, loc. cit., p. 615.

[2] E. F. Smith, Peach Yellows, A Preliminary Report, U. S. Dept. of Agriculture, Botanical Division, Bulletin No. 9, 1888.

for sandal, as only one of the buds formed connection with the healthy stock and even this one died soon after. It was then decided to attempt other methods, and various different methods of grafting, including approach grafting, were tried. A large number of these grafting experiments were successful, and it was soon evident that the disease was being communicated to the orginally healthy stock. The accompanying photographs show some of the results obtained.

Plate XI shows the results of one of the first successful experiments in producing the disease by grafting. As will be seen from Plate XI, Fig. 1, before the application of the scion,[1] the tree was cut back to the stump, only one branch being retained to which the scion, a small piece of twig from a diseased tree, was applied in the form of a cleft graft. To prevent drying out, the point of application of the scion was first bound with plantain (*Musa sapientum*) fibre, and the whole was then covered with ordinary red lateritic clay. In Fig. 1, which was taken sixty days after the date of grafting, we see that that scion has already begun to produce leaves which have the typical spiked character. Fig. 2, taken three months after the grafting, shows further growth of the scion with the production of an abundance of closely crowded leaves. As will be seen, the stock has produced no growth whatever. In Fig. 3, taken one hundred and sixty days after grafting, the scion has grown out into a tuft of spiked branches, very much resembling a witches' broom, while the stock has produced one miserable little twig which, in the picture, can be just made out near the point of attachment of the scion. This twig produced one or two minute leaves typical of an advanced stage of the disease and then died. Shortly after the photograph reproduced in Fig. 3 was taken, the whole top of the tree died.

Plate XII illustrates the results of another interesting experiment. At B in Fig. 1, which is a reproduction of a photograph taken sixty days after the grafting operation, will be seen the spiked scion which was grafted on to the side of the stock and which has already produced typical spiked leaf growth. At A, Fig. 1, is seen another scion attached by means of a cleft graft to another branch of

[1] A scion is a portion of a tree or other plant, usually a short piece of branch bearing healthy buds, which is grafted on to another plant of the same or a related species. This latter is called the stock.

the healthy stock. This particular scion was taken from the same tree which furnished the scion shown at B, but from a portion of the tree which was outwardly still healthy. As will be seen, the growth of A is also apparently quite healthy, the leaves and twigs having a normal appearance. Plate XII, Fig. 2, from a photograph taken three months after the grafting, shows a further growth of both scions in which scion A still retains its normal appearance. In Fig. 3 (photo about nine months after grafting), which was taken just before the death of the tree, we find scion B in the last stages of the disease. Practically all the leaves have dropped off, only a few leaves showing an advanced stage of spike being left at the tips of the twigs. Scion A shows a peculiar phenomenon. Here, instead of new branches breaking out into a spiked growth, all the apparently healthy leaves have dropped off. This happened within the course of two or three days. The branches themselves have, in the picture, been pruned at the ends in an attempt to force the scion to send out new leaves. This, however, was of no avail and the tree died almost immediately after the taking of the photograph. It is interesting to note, in this case, that the originally healthy stock did not send out a single fresh branch.

The above two series of illustrations show extreme effects of grafting in which the healthy stock succumbed, almost without a struggle, to the effects of the infection. The experiment illustrated in Plate XIII and in Plate XV, Fig. 2, shows a more interesting, and at the same time more typical, example of the effects of infection by grafting where the stock has been able to make quite a respectable growth. In this case, as in the other two experiments already described, the healthy stock was cut back severely, there remaining one strong branch and a stump of another, shown to the left in Plate XIII, Fig. 1. This figure reproduces a photograph taken seventy-eight days after the grafting operation. The spiked scion shown at B has made considerable growth and has sent out three branches all of which bear typically spiked leaves. The upper leaves on the left branch of the scion already show an advanced stage of the disease. At A is shown a branch from the originally healthy stock. This has produced beneath a number of quite normal leaves but farther up has broken out into a stage of spike comparable to that

exhibited by the left branch from the diseased scion. To the right of A is to be seen a second branch originating from the stock at the same point as A and this branch, as yet, shows no outward sign of the disease, all the leaves being normal. A branch has also begun to grow from the stump to the left, and this also shows a normal development of leaves. Plate XIII, Fig. 2, taken seventy-two days later, shows a further stage in the development of the infection. Branch A, which in Fig. 1 had begun to show the development of spike, now shows an extensive spiked growth, while on the branch to the right of A the disease has also broken out. Branch C, from the stump to the left, which in Fig. 1 had just begun to grow, has made a vigorous growth and is still outwardly quite free from disease. Plate XV, Fig. 2, which is from a photograph taken seven months and twenty-four days after the grafting operation, shows a very striking change. There has been a very marked growth in all the branches and the whole tree has been infected. The stump to the left has thrown out a number of branches, all of which are now badly spiked. There is scarcely a normal leaf left on the tree. The tree died five months and twenty-four days after this photograph was taken or almost eleven months after grafting.

Plate XIV, Figs. 1 and 2 and Plate XV, Fig. 1, show three stages of the development of the disease from a graft infection on another tree. They require no particular explanation and are given here simply to show the remarkable bush-like growth produced on a healthy stock by this mode of infection. The spiked scion is, in each case, to the right of the picture near the top and, in Plate XIV, Fig. 2, the growth from this scion has been separated from the branched growth from the stock which appears on the left of the picture. As will be seen in this figure, the only normal leaves on the tree are those on branches which have grown out from the stump at the lower right-hand corner of the picture. In plate XV, Fig. 1, which shows a later stage of infection on the same plant, even these branches have become almost fully spiked. A comparatively early stage of a somewhat similar graft infection experiment is shown in Plate II, Fig. 2. The spiked scion is shown at A, while the development of spiked growth on branches proceeding from the stock is very clearly shown.

The illustrations of graft infections, which have been

described above, show cases where the originally healthy stock has been severely cut back, as is customary in grafting, in order to force the sap up into the scion and thus to promote its growth. In subsequent experiments, it has been found possible to get grafts of spiked material to grow without first disturbing, in any serious manner, the normal branch and leaf development of the tree. One such experiment is illustrated in Plate XVI, Fig. 1. As will be seen from the illustration, in this experiment some of the lower branches were pruned back or removed but the top of the tree was left undisturbed. Two secondary branches which had been given off near the base of the stem were pruned back and, to each of them a spiked scion was attached laterally (at A in the figure). These grew out into typical spiked branches and are shown in the figure, which is from a photograph taken one hundred and two days after the grafting operation. The only outward effect on the stock was the production of a very small spiked twig upon one of the branches upon which the grafting was done. This twig, which appeared shortly after the photograph was taken and died soon afterwards, came out at the base of the Y shown in the figure and almost immediately above the point of attachment of the inner scion.

Although there have been no further external evidences of infection on the tree up to the present (March, 1917), there are other signs that the infection has spread. Thus, in August 1916, an examination of twigs from many parts of the tree showed signs of an accumulation of starch and as this was considerably earlier in the year than the period when starch normally appears in the branches of healthy trees, it may be considered as an indication of infection. The tree itself now looks very unhealthy and has not yet shown any sign of putting out a fresh flush of leaves although a large number of the old leaves have already dropped off. No examination of the roots has, as yet, been made as it has been thought desirable to ascertain whether the tree would be killed by the infection, and any examination of the roots might interfere seriously with any resistance to the disease which the tree may be able to exert. A subsequent experiment, at present under way, has shown undoubtedly that the disease may be communicated by grafting on a tree which has been allowed to retain practically all its branches and leaves; so there can be no question of the disease being

due in any degree to the pruning back of the stock. Further evidence, if that were necessary, is given by the fact that, in cases where trees have been cut back and grafted and the scions did not grow, no sign of the disease has appeared.

These experiments have yielded a large amount of valuable information with regard to the disease in addition to the definite establishment of the fact that it is communicable. In the first place, the disease has made its first appearance on the stock almost invariably on branches which lie closest to the point of grafting and has gradually spread from there. In the next place there has been invariably a considerable period before the disease makes its appearance outwardly. In other words, it does not seem to develop rapidly. This period we propose to call the incubation period of the disease. As might be expected, this period varies as is shown by the following data: –

Date of grafting	Date of appearance of disease on stock	Period elapsed
1st July 1915 ...	14th December 1915	5 months 13 days.
9th August 1915 ...	30th January 1915 ..	5 months 21 days.
29th November 1915	15th March 1916 ...	3 months 16 days.
15 ...	13th March 1916 ...	3 months 14 days.

In the third place, internal evidence of the success of the grafting experiment has been found, in all cases, before the external signs of infection have appeared. This evidence has been the accumulation of starch in twigs coming from the originally healthy stock. Lastly, in all cases examined, the infection has spread to the roots and has shown itself there by the death of the end roots and haustoria.

It will thus be seen that we have been able to produce the disease on healthy stocks by the grafting of very small pieces of diseased sandal tissue and that the disease thus produced is in every way similar to the disease as it occurs in nature. It seems hardly necessary to add that there can be no question of the disease having been introduced in a natural way as all the experiments described above have been carried out in the compound of the Agricultural Department Laboratories, which is about six miles from

the nearest infected area. Further, although there are quite a large number of sandal trees in the compound, the disease has occurred only on those trees upon which successful graft infection experiments have been made. As already stated, the cutting back of trees preliminary to grafting has had no effect in producing the disease, as we have a number of such cases where the spiked scions did not grow and in not a single one of these has the disease appeared.

The experiments described above show quite clearly that Spike Disease is not primarily a root disease, as Barber supposed, since it can be communicated to the roots from an infection of parts above ground. Many of the trees which have been killed or are in the process of dying from the disease, as produced by graft infection, have undoubtedly root connections with healthy sandal trees in the neighbourhood, so we hope to be able to ascertain with certainty whether the disease can spread through the ground from root to root. It seems highly probable that this will be found to be the case.

Just what is it that is contained in these small pieces of spiked twigs which, when they are grafted on to a healthy stock, has communicated the disease? Only two sources of infection seem at all probable. Either there is some virus or poison which is able to spread from the living cells of the scion to those of the stock or is carried by the conducting channels, whether sieve tubes or vessels, or else some organism is present which has, up to now, escaped our observation. If the disease is due to an organism, it seems highly probably that it is so small as to be invisible under the microscope. The possibility of a microscopically visible organism escaping notice, while remote, must not, however, be ignored. The beautiful series of investigations carried out in recent years by Smith and his assistants on the organism of Crown Gall[1] show how extremely difficult it is at times to establish the presence of an organism in attacked tissues.

If the infection is due either to a virus or to an ultramicroscopic organism there should exist a possibility of extracting the infectious material by grinding up diseased tissue in water and then filtering off the extract. This infectious material so extracted should then, if injected

[1] Smith, Brown and Townsend, Crown Gall of Plants, its Cause and Remedy, U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin No. 213, 1911.

into a healthy tree, produce the disease. Experiments have been carried out with this end in view. For the purpose fresh spiked material (leaves and twigs) was macerated in distilled water and extracted. The extract was then passed through filter paper to remove solid particles. Part of the extract thus obtained was passed through a Chamberland filter candle to remove any microscopic organisms present, part was boiled to destroy possible ultramicroscopic organisms or a virus that might be present, and part was left untreated. Injection was attempted in the first place by means of glass tubes similar to those used by Hutchinson[1] in his experiments on the Rangpur Tobacco Wilt. These tubes were bent at right angles at the lower end and drawn out almost to a point. The pointed end was then inserted into a small hole made through bark of the tree as far as the wood and sealed with plasticine. The tube was then filled with the extract. There was considerable difficulty in making a watertight connection but undoubtedly a certain amount of the extract made its way inside. The experiments were tried on young trees in 1916. The results have been, in all cases, entirely negative as all the trees have remained quite healthy.

In a second series of experiments, the bark of young trees was pierced by a circle of small holes, a cup of wax was moulded about the stem immediately below the punctures and the cup was filled with the extract. Here again the results were negative in all cases although there was ample evidence that the extract had penetrated into the trees. In one case the operation caused all of the leaves above the circle of punctures to wither and drop off, due no doubt to the soaking up of the extract. The tree, however, recovered and although the experiment was carried out more than a year ago, has shown no sign of the disease. Other experiments are under way, in which it is being attempted to make healthy trees take up large quantities of the extract and it is hoped by means of these to reach some definite conclusion as to the possibility of producing the disease by means of an extract from diseased trees. All the experimental evidence so far obtained points away from the possibility.

[1] Hutchinson, Rangpur Tobacco Wilt, Memoirs of the Department of Agriculture in India, Bacteriological Series, Vol. I, No. 2, p. 78.

Reference has already been made to the fact that other plants in South India show symptoms in many respects similar to Sandal Spike and one of the lines of investigation has had as its object the ascertaining whether these symptoms are also the result of a communicable disease. In the case of the spike of *Zizyphus œnoplia* already referred to communicability has recently been demonstrated as the accompanying illustrations on Plate XVII show. In Plate XVII, Fig. 1, the scion from a spiked plant of *Zizyphus* is shown at A. At B is shown a branch from the originally healthy stock which bears quite normal leaves. This branch had been produced on the stock before the grafting operation. Since the photograph was taken this branch has shed its normal leaves and has begun to produce spiked ones instead. At C is shown a tuft of leaves from the stock which exhibit the same stage of spike as that shown by the leaves arising from the scion above.

Plate XVII, Fig. 2 shows the results of a similar experiment. Here two scions have been inserted but only one of them, that to the left, has formed connection. This scion has produced two tufts of typically spiked leaves. The stock shows, at B, a similar tuft. Within the past few days, or about five months after the grafting was done, the scion has died but the stock is still producing fresh spiked leaves vigorously.

Recently, similar graft infection experiments on *Stachytarpheta indica* have given definite results also, as is shown in the accompanying illustration (Plate XVIII). One of the striking symptoms of the disease in this plant is phyllody. As will be seen, the diseased scion attached at A exhibits this to a marked degree at B. The stock had originally put out quite healthy flower spikes, one of which is shown at D. Since the grafting, spikes have been produced on the stock which show the same abnormal phyllody as was shown by the scion.

As is the case with the two *Zizyphus* plants described, this plant is still growing and we shall have to wait for some time before we can say whether the graft infection will cause the death of the originally healthy stock as it has done in similar experiments with sandal. In any case, the early symptoms of graft infection are in these two species practically identical with those obtained in the case of sandal. Similar infection experiments with *Vinca rosea* are under way.

The next step in this part of the investigation is to ascertain whether the disease as it occurs on *Zizyphus* or *Stachytarpheta* can be communicated to sandal and *vice versa*. It may be pointed out here that *Zizyphus* spike appears to be much more widely spread than sandal spike and is present in areas where the latter disease has not yet appeared. Cross inoculation experiments by grafting would seem to be out of the question and, as has already been pointed out, it has not yet been found possible to produce the disease on sandal by injection, so the difficulties in the way of carrying out this experiment are many. It is proposed to try the experiment of infecting *Zizyphus* plants with which sandal trees have formed haustorial connection and this should give us the information we desire.

Reference has already been made to the fact that diseases in many ways similar to Spike have been found on plants in other parts of the world, and while it would be out of place in a preliminary report such as this to discuss in detail the results of investigations on these diseases, the work done upon them has so important a bearing on the disease we are investigating as to warrant a reference to it.

Among the most important of these diseases are two affecting the Peach Tree in America, Peach Yellows and Peach Rosette. Both of these diseases formed subjects of investigation by Dr. Erwin F. Smith, of the United States Department of Agricultre, for many years, and the results he obtained should be of interest to workers in India.

The diseases are both characterised by a forcing of the growth of branches and leaves resulting from a development of dormant and winter buds. This is accompanied in both cases by a diminution in the size and a premature yellowing of the leaves. In Peach Yellows, there is also a premature ripening of the fruits. Both diseases may appear on one or two branches at first and then spread to the whole tree. Both are fatal, Peach Rosette usually killing the tree within six months while

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The results of Dr. Smith's investigations are given in the following publications :—

i. Peach Yellows, A Preliminary Report, U. S. Department of Agriculture, Botanical Division, Bulletin No. 9, 1888.

ii. Additional Evidence on the Communicability of Peach Yellows and Peach Rosette, U. S. Department of Agriculture, Division of Vegetable Pathology, Bulletin No. 1, 1891.

iii. Experiments with Fertilizers for the Prevention and Cure of Peach Yellows, 1889-92, U. S. Department of Agriculture, Division of Vegetable Pathology, Bulletin No. 4, 1893.

Peach Yellows may take several years. In both the roots may appear normal but the rootlets are dead.

While Dr. Smith has not, as far as I have been able to ascertain, published the results of his microscopic investigations, no mention is made in any of the above reports to an accumulation of starch in either leaves or twigs nor do the results of chemical analyses reveal such striking differences in composition as are given above for Spike Disease of Sandal, although differences do occur.

The immense mass of evidence collected shows conclusively that these diseases cannot be considered as due to unfavourable climatic or soil conditions or to insect or fungus attack. The results of budding demonstrate that the disease can be communicated readily by this means; but there is no reference by Smith to any attempt to communicate the disease by the injection of an extract obtained from diseased tissue.

Another disease of apparently similar nature is the Mosaic Disease of Tobacco. This disease has been investigated by a large number of scientific workers and the most varied conclusions have been reached with regard to its cause. The latest work on the subject is that by Allard.[1] This author has confirmed the views of Beijerinck and others that the disease is transmitted by a specific infective material or virus and can be readily communicated by means of injection of the juice of a diseased plant into a healthy one. Allard has shown clearly that the virus is something quite distinct from the ordinary enzymes or ferments produced in the healthy tobacco plant. The fact that he was able to remove the virus or at least to destroy its infective properties by passing it through a bacterial filter would seem to indicate that the so-called virus really contains an organism so minute as to be invisible under the miscroscope, and in fact Allard comes to this conclusion, as is shown by the following quotation :—" A specific particulate substance not a normal constituent of healthy plants is the cause of the disease. Since this pathogenic agent is highly infectious and is capable of increasing indefinitely within susceptible plants, there is every reason to believe that it is an ultramicroscopic parasite of some kind." In a subsequent

[1] H. A. Allard, The Mosaic Disease of Tobacco, U. S. Department of Agriculture, Bulletin No. 40, 1914 ; Some Properties of the Virus of the Mosaic Disease of Tobacco, Journal of Agricultural Research, Vol. VI, p. 649, 1916.

paper Allard has described a disease of *Nicotiana viscosum* which closely resembles the mosaic disease of tobacco but which is nevertheless distinct.[1] Thus, while ordinary tobacco (*Nicotiana tabacum*), *Nicotiana rusticum*, tomatoes (*Lycopersicum esculentum*) and *Capsicum cerasiforme* are readily infected by the virus of the common mosaic disease, the virus of the disease of *Nicotiana viscosum* has, when inoculated into them, given no results.

Another interesting feature of this disease lies in the fact that it is not so readily transferred by needle inoculations as is the ordinary form of mosaic disease. He found in cases where attempts were being made to communicate the disease to hybrids of *N. tabacum* ♀ and N. *viscosum* ♂ that the disease could be much more readily communicated by grafting than by needle inoculations. This phenomenon will be referred to later.

Another disease which appears to bear some resemblance to Spike Disease is the Leaf Roll Disease of Potatoes, which has been under investigation for a long time in Europe, more especially in Germany and Austria. Various theories have been advanced as to the cause of this disease, among them being deterioration of potato varieties, soil and climatic conditions and fungus attack. Recent researches by Quanjer,[2] however, appear to make it probable that the disease is due to a transmissible virus. To quote from *Nature*, "Since attempts made to infect healthy potato plants by means of injections of the sap of diseased plants did not succeed, it might be thought that the claim is not justified. However, successful transmission of the disease was brought about in grafting experiments both with stalks and with tubers. Further evidence in favour of the virus is claimed to be afforded by the failure to isolate any parasitic organisms from infected plants, by the method of spread of the disease, by the uncertain results of selection as a means of raising healthy stocks of plants and by the infection of healthy plants when transferred to diseased surroundings either through the agency of the soil (in which it is believed that the virus is often present) or from neighbouring diseased plants." I have unfortunately not had access to

[1] H. A. Allard, A Specific Mosaic Disease in *Nicotiana viscosum* Distinct from the Mosaic Disease of Tobacco, Journal of Agricultural Research, Vol. VII, p. 481, 1916.

[2] Quanjer, Mededeelingen van d. Rijks Hoogere Land, Tuin, en Boschbouwschool, Wageningen, Diel X, 1916; cited from *Nature* Vol. 98, p. 500, 1917.

Quanjer's paper, but the above comments indicate a distinctly similar condition of affairs to that obtaining in Sandal Spike.

A recent paper by Boncquet[1] on the "Presence of Nitrites and Ammonia in Diseased Plants," has also a bearing on the question. This author, in a previous paper on the Curly Leaf of the Sugar Beet, had found a specific bacterium inhabiting the sieve tubes of sugar beets affected with the disease known as Curly Leaf. The same bacterium was found in connection with various other foliage abnormalities of the same plant. This organism proved to be a vigorous nitrate reducer and, wherever the organism was present, nitrites or ammonia were found in the affected plants, whereas in normal plants no trace of nitrites or ammonia could be made out.

This author claims to have found a very minute bacterial organism, a streptococcus in the virus of mosaic disease of tobacco and a similar organism in the case of certain obscure diseases of the potato. These organisms also proved to be nitrate reducers. Similarly in a number of other plants the presence of nitrites was found associated, on the one hand, with "abnormal development from a structural standpoint, like the deformities of leaf, dwarfing, curling and distorting," and, on the other, with the presence of bacteria which were found to be active nitrate reducers. According to this author the plants attacked, although growing in a soil rich in nitrogen, show all the symptoms of nitrogen starvation, and he attributes this to the invasion of the tissues by nitrate-reducing organisms which decompose the nitrates to nitrites or ammonia and so render the nitrogen unavailable for the plant.

Shear,[2] in a recent Bulletin of the U. S. Department of Agriculture, has described a disease of the cultivated cranberry (*Oxycoccus macrocarpus*) which bears a striking resemblance to Spike Disease. This disease, which is known as "False Blossom," is characterised by a striking phyllody, and an excessive vegetative growth with the development of lateral branches from the usually latent axillary beds. The development of these branches gives

[1] Boncquet, Presence of Nitrites and Ammonia in Diseased Plants, Journal of the American Chemical Society, Vol. XXXVIII, p. 2572, 1916. See also Ralph E. Smith and P. A. Boncquet, New Light on Curly Top of the Sugar Beet, Phytopathology Vol. 5, pp. 103-107, 1915 and Connection of a Bacterial Organism with Curly Leaf of the Sugar Beet, Phytopathology, Vol. 5, pp. 335-342, 1915.

[2] C. L. Shear, False Blossom of the Cultivated Cranberry, U. S. Department of Agriculture, Bulletin No. 444, 1916.

the plant a kind of witches'-broom appearance. The author also states that plants attacked by the disease rarely develop normal fruit. The cause of the disease has not been ascertained, and while there are indications that it is infectious in nature this has not been established. Shear considers that it may be due to unbalanced nutritive conditions and states that there is at present no evidence to indicate that it is produced by insects or fungi.

As has been noted, all the above-mentioned diseases show many symptoms similar to those of Spike Disease. In the case of none of them, however, is there any mention by investigators of excessive starch accumulation, and as far as I have been able to ascertain, this symptom is peculiar to the various spike diseases which we have investigated in Mysore. While a virus cause cannot be considered as definitely established in all the diseases enumerated, it is interesting to note that there appears to be a gradation in the infective power of the virus by direct inoculation. On the one hand, we have the Mosaic Disease of Tobacco which is very readily transmissible by needle inoculations ; on the other hand, diseases such as Spike Disease of Sandal, Peach Yellows and Peach Rosette and Leaf Roll of Potatoes which appear to be transmissible only by grafting. Midway stands the disease of *Nicotiana viscosum* in which infection by means of inoculation with the juices of diseased plants is still possible but is not so readily accomplished as is infection by grafting.

The fact that Spike Disease of Sandal is communicable leads to the important question of its mode of spread. The spread of the disease in areas already infected is, as already stated, comparatively easy to understand as its communication by means of the roots is quite possible. On the other hand, where we find it appearing in isolated areas separated from each other by many miles, this method of infection cannot come into play. While we cannot, with our present knowledge, state just what agencies are at work in spreading the disease over long distances, certain possibilities present themselves.

In the first place there is the possibility of spread by means of seed. As already noted, trees in an advanced stage of the disease never form seed but it is not uncommon to find seed formation on the outwardly healthy

portions of partly attacked trees. Furthermore it is quite conceivable that a tree recently infected might produce abundant seed before it showed any outward signs of disease at all. We do not yet know whether such seeds actually carry the virus or, if they do, whether they are able to produce seedlings which can establish themselves in the ground. Experiments to settle this question are in progress, and until the contrary has been proved we must consider diseased seed as one of the possible means of spread. To what extent seed collected in diseased areas is transferred to areas still free from disease, I am not in a position to state, but it seems advisable to warn Forest Departments not to allow any such transfer until it has been definitely proved that the disease cannot be communicated in this way.

Another possible means of spread is by birds or insects. Birds might act as carriers of diseased seed, while insects might conceivably convey the virus from tree to tree over considerable areas. Sucking insects which live on the juices of the sandal tree are the ones most to be suspected, and a number of these are known. It is true, that, up to the present, we have not been able to communicate the disease by any form of inoculation, but it is possible that further experiments in this direction may meet with success.

The fact that several other species of plants which grow in sandal areas show symptoms very similar to Spike Disease makes it possible that these species may contribute to its spread. As already pointed out, the Spike Disease of *Zizyphus œnoplia* appears to be much more widely spread than sandal spike, so *Zizyphus* and the other attacked species must be looked upon with suspicion as possible sources of infection.

Finally we have to consider the view that the disease where found in isolated areas has arisen *de novo*. To admit this view as possible would be to admit that the production of the virus results from unfavourable conditions which upset the normal metabolism of the sandal tree and that the virus when thus formed is able to multiply and to infect healthy plants in areas where such unfavourable conditions do not exist. While views similar to this have been advanced by investigators to account for a number of obscure so-called physiological plant diseases such views do not rest on a sound foundation. It

seems to me highly probable that the cause of every really infectious plant disease will be eventually traced to some specific organism or organisms and, in fact, we find as time goes on more and more diseases taken out of the physiological class and placed among those proved to be due to parasitic action.[1]

Conclusions.

1. Sandal Spike is a very serious disease which has already spread over large portions of the sandal-growing area of South India. It is at present responsible for losses which may be estimated at between five and six lakhs a year, while the possibilities of damage from it in the future are enormous.

2. There is no satisfactory evidence to show that the disease is caused by unfavourable conditions whether of soil or climate or by association with unsuitable host plants or by overcrowding. On the other hand it seems probable that, given the causative agent, the presence of unfavourable conditions increases the virulence and hastens the spread of the disease.

3. The disease is marked by a profound disturbance of the ordinary life processes of the Sandal tree which leads to a continuous growth of leaves and twigs and a gradual reduction in size of the latter. Two of the most striking symptoms are the accumulation of starch in leaves and branches and the death of the haustoria and the root ends.

4. The chemical composition of the leaves is markedly altered by the disease and their disastatic activity is seriously diminished.

5. There is no satisfactory evidence to show that either fungus or insect attack can be considered as a cause of the disease.

6. The disease is communicable by grafting and is in all probability produced by a virus whose active principle is an ultramicroscopic organism. The fact, now for the first time established, that the disease is infectious, is of the highest importance. With the uncertainty on this point that has existed in the past it was hardly to be expected that combative measures would be carried out in

[1] *Vide* R. E. Smith, The Investigation of Physiological Plant Diseases, Phytopathology, Vol. 5, p. 83, 1915.

every case with the greatest vigour. Officers who believed that the disease is the result of unfavourable external conditions could scarcely be expected to carry out enthusiastically combative measures which were based on the supposition that the disease is infectious. In the future there will hardly be an excuse for a sceptical attitude on this point and the greatest vigour should be displayed in preventing the spread of the disease by removing promptly all diseased trees in newly infected areas. In a tract where the disease has gained a firm foothold and has embraced a large area, this is, of course, impracticable, and the best that can be done in such cases is to try to check its spread on the borders of the infected area. Favourable results seem to have been obtained in Coorg with work on these lines. With an extension of our investigations it seems probable that we shall be able to establish more efficient and less costly means of combating the disease but until that has been accomplished removal operations on the lines laid down should be continued.

7. A number of other species of plants showing similar diseases have been found in areas where sandal grows. Some of these diseases have been shown to be infectious in the same way as sandal spike, and it is possible that the same cause which produces sandal spike is active in these diseases also.

8. The means of spread of the disease are probably varied. Direct attachment of the roots of healthy trees to those of diseased ones, or *vice versa*, no doubt accounts for much of the spread of the disease in infected areas. Other possible means of spread are the carrying of seed produced on diseased trees either by man or birds, the carrying of the virus by means of insects and the infection of sandal from other plants attacked by a similar disease.

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PLATES

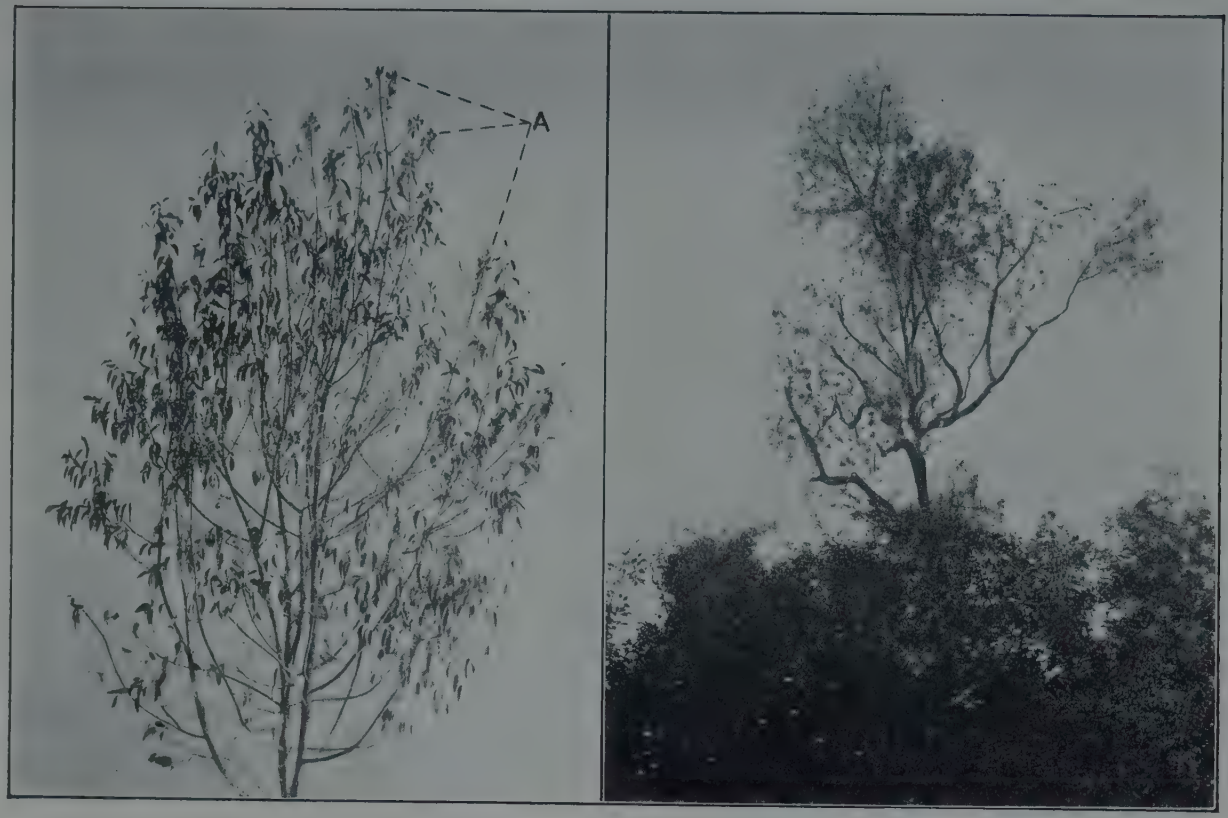

Plate I.

FIG. 1.

Young Sandal Tree showing early stages of Spike Disease. The twigs at A show spiked condition.

FIG. 2.

Sandal Tree in last stages of Spike Disease.

FIG. 1.

A, Inflorescence from healthy sandal tree; B, healthy sandal flower; C, inflorescence from diseased tree, showing phyllody and D, individual flower showing phyllody.

FIG. 2.

Graft infection experiment. Grafting done 29th Nov., 1915. Photo., 4th May 1916. Point of attachment of graft shown at A.

Plate II.

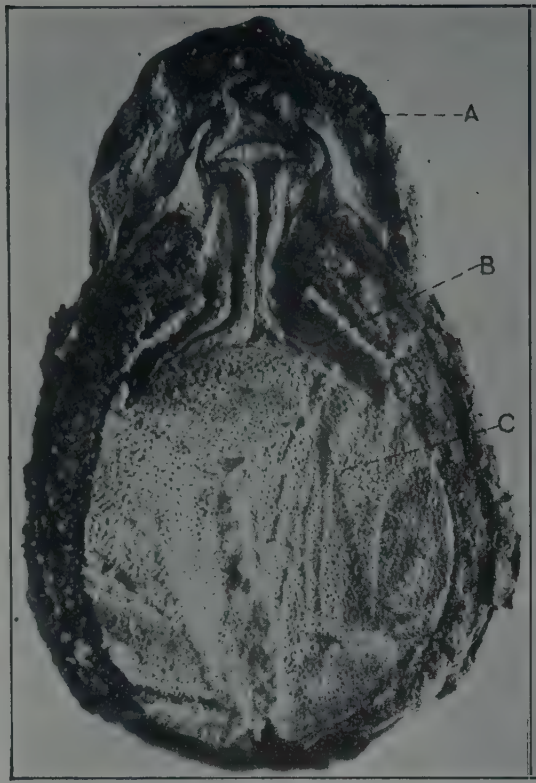

FIG. 1.

Sandal haustorium on Lantana root. A, top of haustorium; B, penetrating sucker of haustorium applied to wood of host root; C, wood of host root.

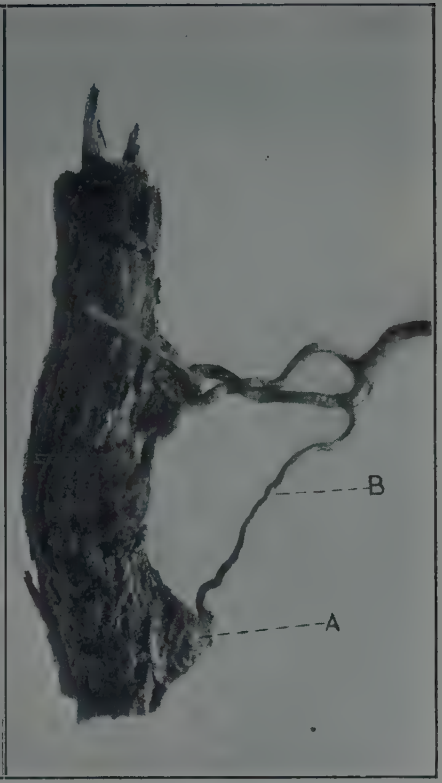

FIG. 2.

Sandal haustoria on root of *Vitex negundo* A, haustorium; B, root from which haustorium has arisen.

Plate III.

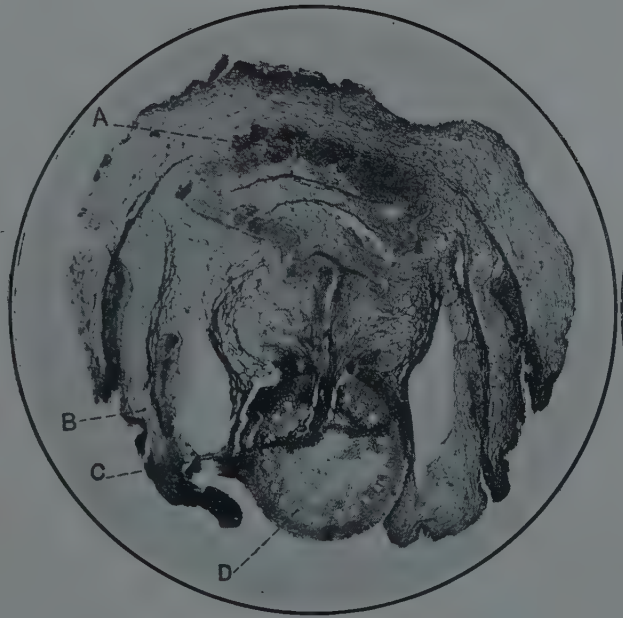

FIG. 1.

Cross section of grass root attacked by Sandal haustorium. A, vascular bundles of mother root; B, lateral fold of haustorium; C, penetrating sucker of haustorium applied to vessels of grass root; D, grass root in cross section.

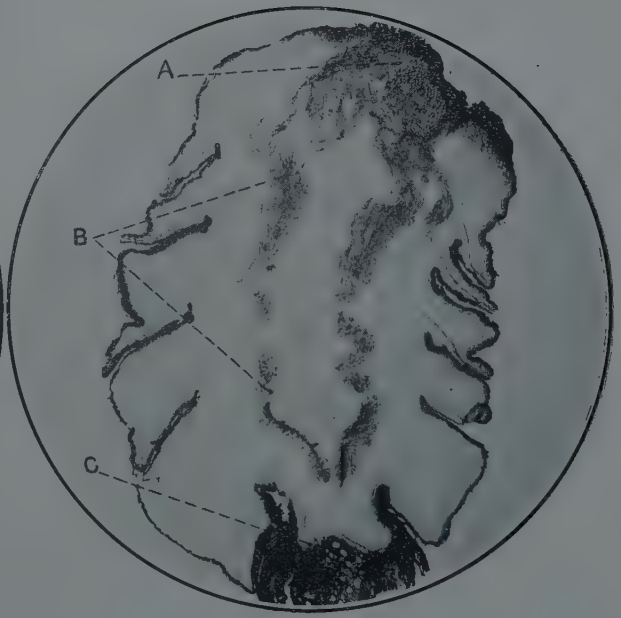

FIG. 2.

Sandal haustorium attacking root of *Cassia auriculata*. A, central vascular bundles of mother root; B, vascular strands leading to host root; C, sucker applied to wood of host root.

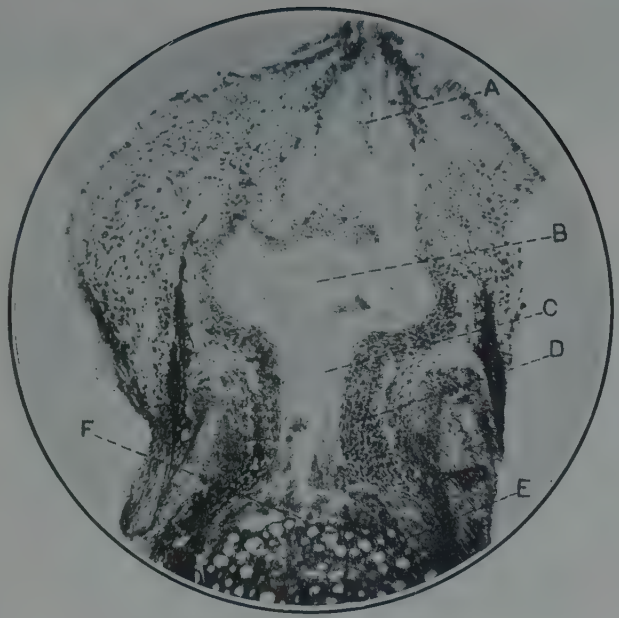

FIG. 1.

Healthy Sandal haustorium on root of Lantana. A, central vascular bundles of mother root; B, lacunar tissue; C, vascular strands passing down to host root; D, cortex of haustorium loaded with starch grains; E, host root; F, sucker at point of attachment to host root.

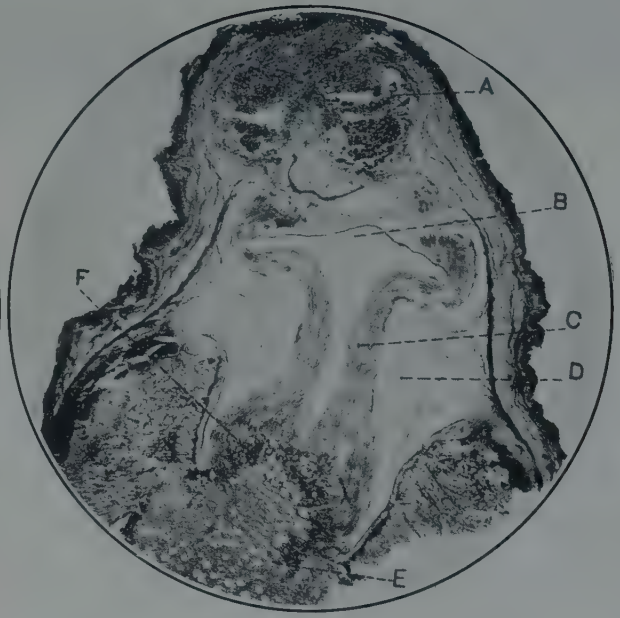

FIG. 2.

Haustorium of tree attacked by spike attached to root of another Sandal tree. Letters as in previous figure. Note that here cortex of haustorium contains no starch grains.

Plate V.

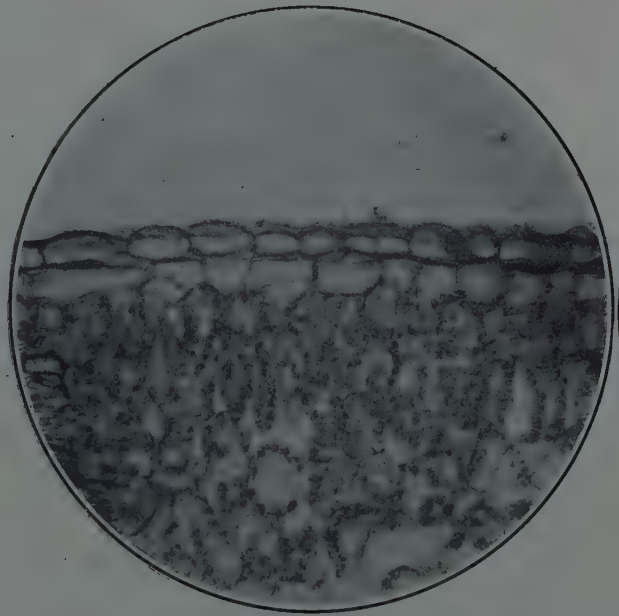

FIG. 1.

Section of a spiked leaf taken on a panchromatic plate. The chloroplasts are brought out distinctly, but the starch grains embedded in them are indistinct.

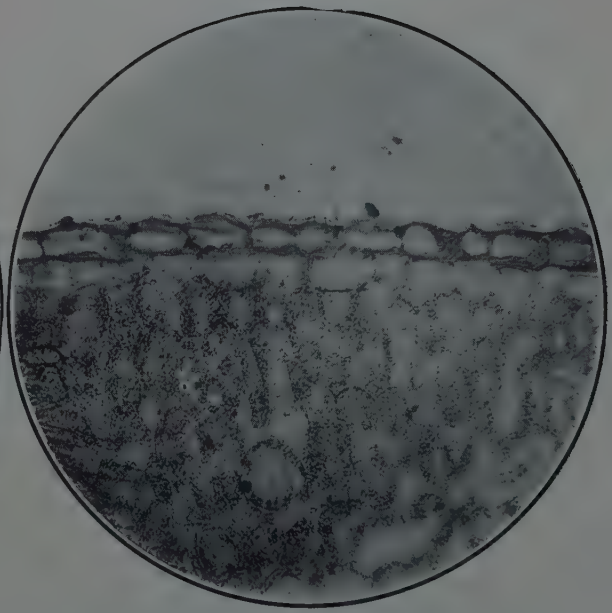

FIG. 2.

Same section taken on an ordinary plate. Here the chloroplasts cannot be made out, but the minute starch grains come out distinctly as small black dots. Section stained according to Nemec's gentian violet method.

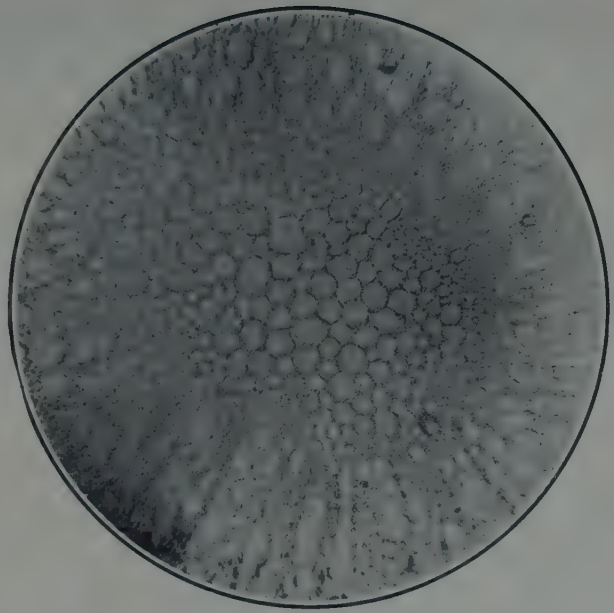

FIG. 1.

Cross section of a healthy Sandal twig, showing absence of starch in pith and medullary rays.

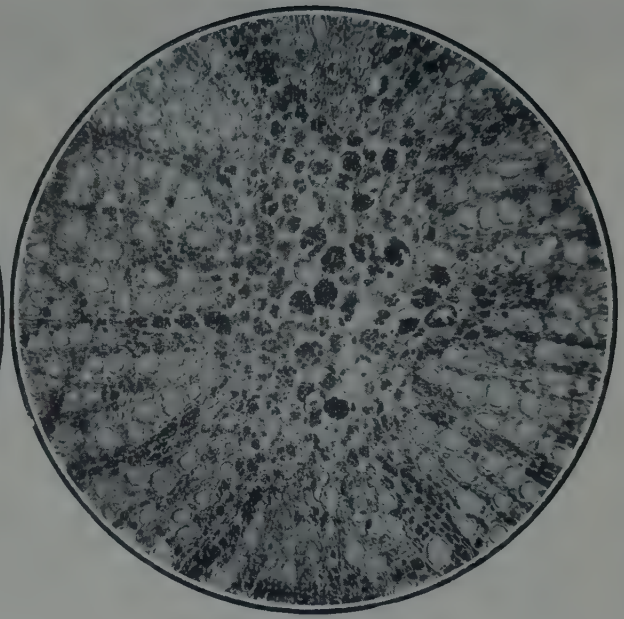

FIG. 2.

Section of Spiked Sandal twig, showing abundance of starch in pith and medullary rays. Section stained with iodine in potass. iodide.

Plate VII.

Zizyphus œnoplia. A, healthy twig showing normal leaves; B, spiked branch showing reduced leaves.

Plate VIII.

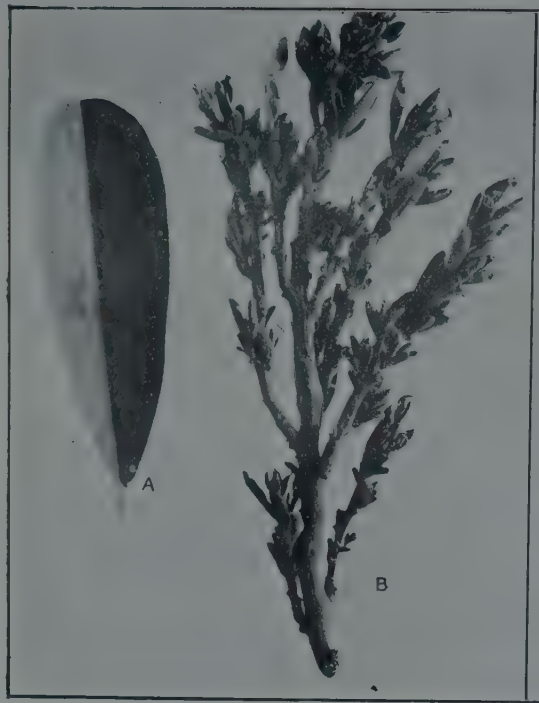

FIG. 1.

Crotolaria retusa. A, healthy leaf; B, spiked branch showing reduced leaves.

FIG. 2.

Vinca rosea. A, healthy branches and leaves; B, spiked branches and leaves.

Plate X.

FIG. 1.

Plant of *Datura stramonium*, showing spiked growth at base.

FIG. 2.

Another specimen of *Datura stramonium*, showing similar spiked growth.

FIG. 1. Graft infection experiment. Grafting, 1st July, 1915. Photo. 30th Aug., 1915.

FIG. 2. Later stage. Photo. 1st Oct., 1915.

FIG. 3. Later stage. Photo. 14th Dec., 1915.

Plate XI.

FIG. 1.

Graft infection of Sandal. Grafting done on 1st July, 1915. Photo. 30th Aug. 1915. A, normal scion; B, spiked scion.

FIG. 2.

Later stage of same experiment. Photo. 1st October, 1915. Lettering as in Fig. 1.

FIG. 3.

Later stage of same experiment. Photo. 29th March, 1916. Lettering as in Fig. 1.

Plate XII.

FIG. 1.

Graft infection experiment. Grafting done 29th Nov., 1915. Photo. 15th Feb., 1916. A, branch from stock showing spiked growth at apex; B, spiked scion.

FIG. 2.

Later stage of same experiment. Photo. 27th April, 1916. A and B, as in Fig. 1; C, healthy branch growing from stump.

Plate XIII.

Plate XIV.

FIG. 1.

Graft infection experiment. Grafting done 9th Aug., 1915. Photo, 14th Feb., 1916.

FIG. 2.

Later stage of same experiment. Photo, 9th Mar., 1916.

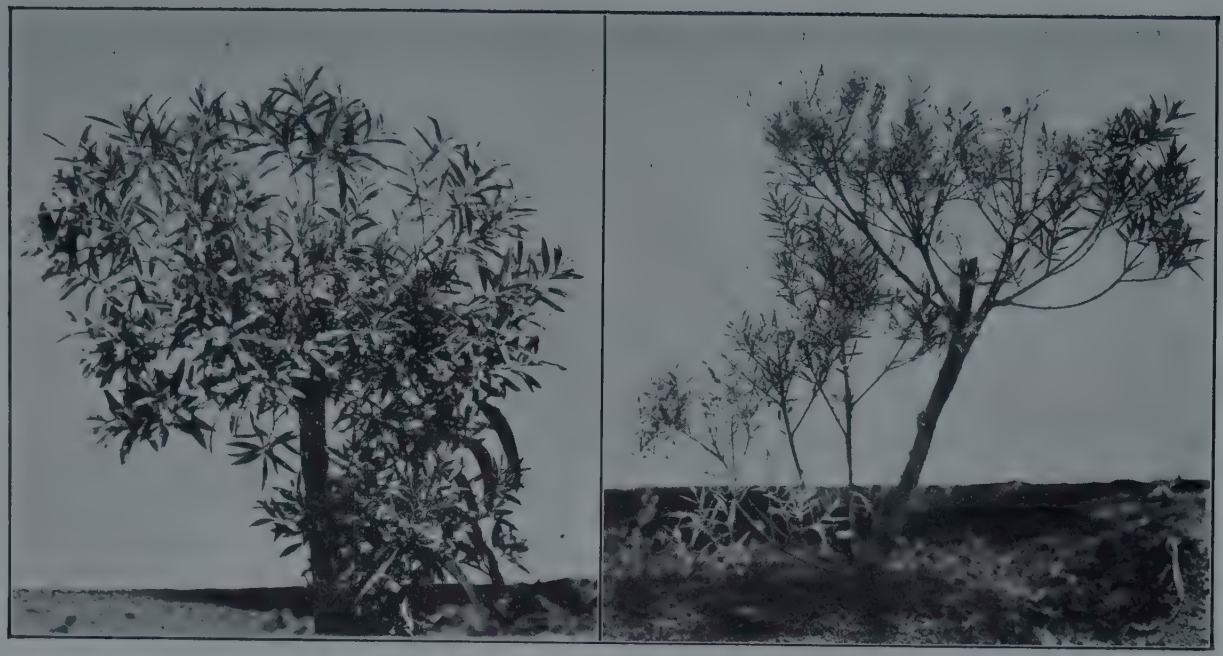

FIG. 1.

Later stage of experiment illustrated on Plate XIV. Photo. 29th Mar., 1916.

FIG. 2.

Later stage of experiment illustrated on Plate XIII. Photo. 6th July, 1916.

FIG. 1.

Grafting experiment on healthy Sandal tree. At A are seen two scions from a spiked tree which have already made considerable growth. Grafting done on 29th Nov., 1915. Photo. 11th April, 1916.

FIG. 2.

Lantana root with a number of Sandal haustoria attached. Some are shown at A.

FIG. 1.

Graft infection experiment on *Zizyphus œnoplia*. Grafting done on 21st July, 1916. Photo. 9th Jan., 1917. A, spiked scion; B, healthy branch from stock; C, spiked branch from stock.

FIG. 2.

Similar experiment. Grafting done on 21st July, 1916. Photo. 8th Jan., 1917. A spiked scion; B, spiked branch from stock.

Graft infection experiment on *Stachytarpheta indica*. Grafting done on 21st July, 1916. Photo. 18th Jan., 1917. A, point of insertion of scion; B, flower spikes of scion showing phyllody; C, flower spikes of stock showing phyllody; D, normal flower spike of stock with flowers shed.

Plate XVIII.